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BLACK CHAFF DISEASE OF WHEAT[1]

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INTRODUCTION

Black chaff of wheat (*Triticum aestivum* L., *T. vulgare* Vill.), caused by *Bacterium translucens* var. *undulosum* Smith, Jones, and Reddy, has been present in the wheat-growing region of the Mississippi Valley for a number of years. Although it has become distributed generally throughout the central and upper Mississippi Valley, it usually does not become epidemic over large areas on the varieties commonly grown. This sporadic occurrence of severe infection may be due to variations in environment. No extensive investigations have been made to determine what environmental conditions favor the development of epidemics of the disease, although the heaviest infections have occurred in wet seasons. In the hard red spring wheat area Hope and H–44, two varieties that have proved very resistant to stem rust, are being used extensively as parent stock in the breeding of new disease-resistant varieties. Since these two varieties are among the most susceptible to black chaff, there is danger of the disease becoming epidemic under favorable environmental conditions should selections obtained from crosses with these varieties, or with other susceptible ones, become widely grown. Therefore, it is evidently desirable to know the susceptibility or resistance of the new varieties and hybrid selections grown. However, in order to test most effectively varieties and hybrid selections for their reaction to black chaff by means of artificial epidemics, it is necessary to know the environmental conditions most favorable for the production of an epidemic and the life history and physiologic specialization of the pathogen.

GEOGRAPHIC DISTRIBUTION AND ECONOMIC IMPORTANCE

The black chaff disease of wheat is widely distributed, occurring rather generally in many of the principal wheat-producing countries of the world. In North America it is known to occur in the United States, Canada, and Mexico. The first record of its presence in the United States was probably made by Heald (*10*)[3] in 1906, when he

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[2] The writer gratefully acknowledges his indebtedness to the following members of the staff of the Minnesota Agricultural Experiment Station: Dr. E. C. Stakman, under whose direction these investigations were made, Dr. J. J. Christensen for suggestions and criticism during the progress of the work, and Dr. J. G. Leach for help in the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 416.

briefly called attention to a bacterial blight of the leaves of wheat in the breeding plot at the Nebraska Agricultural Experiment Station that year. Since that time it has been found throughout the Mississippi Valley and in California (*19*).[4 5] Bisby and Buller (*2*) first reported the presence of black chaff in Canada in 1922. Drayton (*5*) stated that it occurred there in 1923 and 1924, and Greaney (*8*) found it in Manitoba every year from 1926 to 1929. During the summer of 1931 the writer received from Mexico specimens of wheat affected by a disease which he identified as black chaff. The specimens were collected by H. G. Ukkelberg in the State of Coahuila. Other specimens were received in April 1933 from Wallace Butler from the State of Nuevo León.

Jaczewski (*12*) first reported black chaff in Europe in 1925. He found it on wheat samples collected as early as 1910 and 1911 from the Provinces of Mohilev in Belorossia and Elisavetopol in Crimea. He also found it on samples from the Province of Poltava in Ukraine collected in 1916, from Crimea in 1922, and from Kharkov and Kiev in 1924. It was also found in the Provinces of Voronezh and Don, and the district of Kuban in Caucasia. Millasseau (*16*) first noted the disease in France in 1927. He found it rather generally distributed in the area around Paris. The next year (1928) Hocquette (*11*) found it at Cappelle, in the Department of Nord, which is adjacent to Belgium, and Marchal (*15*) found it in Belgium in 1929.

Jaczewski (*12*) gave evidence of the occurrence of the disease in Asia as early as 1893. He found black chaff on specimens of wheat collected in Sungpan, China, in that year. No other reports of the disease in Asia have been found.

The first report of the occurrence of black chaff in Africa appeared in 1930. Verwoerd (*22*) found a bacterial disease on wheat in the plots at the College Cereal Experiment Station, Malmesbury, South Africa, in 1929, which he later identified as black chaff. Burton (*4, Rept. 1930*) noted the occurrence of the disease in Kenya, British East Africa, in 1930.

Published data regarding the losses in yield and quality of grain due to black chaff are limited. The reports that have appeared have mostly been estimates of losses over large areas without experimental data giving comparative yields of infected and uninfected plants. Burton (*4, Rept. 1931*) reported that a number of varieties in yield trials in 1931 were completely destroyed by the disease. Waldron (*23*) made a comparison between the weight of grain from infected and noninfected plants in a number of F_3 families from crosses of Hope with other varieties. He found that there was a decrease in the weight of the grain from the infected plants and that as an average of several families the decrease was 10.5 percent. Evidently, the amount of loss that would be caused by the disease depends upon the time and severity of infection. The writer has found that where there is severe infection part of the spikelets are often barren and the more severely infected culms may be entirely barren.

HISTORICAL REVIEW

As early as 1906, Heald (*10*) recorded the occurrence of a bacterial blight of wheat in the breeding plot at the Nebraska Agricultural

[4] SMITH, E. F. BLACK CHAFF OF WHEAT. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Survey Bull. 2: 98-99. 1918. [Mimeographed.]

[5] HASKELL, R. J., and WOOD, J. I. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1928. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bull. Sup. 71: 275-276. 1929. [Mimeographed.]

Experiment Station. Although he gave no description of the diseased except to say that it was a bacterial blight of the leaves, it probably was black chaff. Jones, Johnson, and Reddy in 1916 (*13*) called attention to a bacterial blight of wheat, which they found to be similar to or identical with a disease of barley that they called bacterial blight. They stated that the symptoms of the two diseases and the cultural characters of the causal organisms appeared to be identical. Although their description of the symptoms of barley blight was brief, it was accurate enough to leave no doubt that the disease which they found on wheat was the same as that later described by Smith (*19*) as black chaff.

Smith (*19*), in 1917, described the disease very clearly as it appears on the glumes of wheat, especially on ripening grain, but he did not point out definitely the symptoms on seedlings or on the parts of older plants other than the glumes. In a note written later in the same year he reported that he had been able to obtain a high percentage of seedling infection with artificial inoculations and that he had isolated the pathogen from diseased leaves, stems, glumes, and kernels.[6] He found bacteria in great numbers in corroded cavities in the interior of shriveled grain and he believed that the general appearance of the pathogen in and on the wheat grains suggested that the organism might live over from one year to the next on infected seed. The following year (1918) he obtained what he considered evidence substantiating this belief and suggested the following methods of ridding the seed of this hold-over inoculum:[7] (1) Screening to remove the shriveled grain containing the bacterial cavities and (2) treating the seed grain with 1:1,000 copper sulphate solution to destroy the surface bacteria.

Braun (*3*) presented very strong evidence that seedling infection occurred in Kansas in 1919 in fields planted to seed from infected fields, and that the seedling infection there was greatly reduced or entirely eliminated by treating the infected seed with formalin or copper sulphate.

Although the bacterial nature of the black chaff pathogen had been known for several years, no description of the organism or its host range was available before that given by Smith, Jones, and Reddy (*20*) in 1919. In morphology, physiology, and cultural characteristics they found it to be almost identical with *Bacterium translucens*, the pathogen of bacterial blight of barley, as described by Jones, Johnson, and Reddy (*14*). The principal difference between the wheat and barley organisms was in pathogenicity. In cross inoculations on the leaves of seedlings the barley pathogen was either noninfectious on wheat or produced small nontypical lesions, while the wheat pathogen was almost as pathogenic on barley as on wheat and the lesions produced on barley by the two organisms were indistinguishable.

OBJECTS OF THE INVESTIGATIONS

The specific objects of these investigations were to study: (1) The factors which contribute to the production of an epidemic, (2) the life history of the causal organism, (3) the method of overwintering

[6] SMITH, E. F. BLACK CHAFF OF WHEAT. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Survey Bull. 1: 40. 1917. [Mimeographed.]
[7] SMITH, E. F. See footnote 4.

of the organism, (4) varietal susceptibility, and (5) the specificity of pathogenicity of the causal organism.

MATERIAL AND METHODS

In the fall of 1929, a culture of the pathogen of black chaff of wheat was obtained from Dr. Delia Johnson, who had made the isolation from diseased wheat grown at University Farm, St. Paul, Minn., in 1929. For convenience this isolate has been called culture M. Another culture was received from A. G. Johnson, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C. This was designated as culture W.

During the early summer of 1931 sectors appeared in old colonies of cultures M and W growing on potato-dextrose agar in Erlenmeyer flasks. These sectors differed from the original colonies in color, viscosity, and rate of growth. Transfers were made from these sectors by removing a small amount of bacterial growth from the margin to fresh agar slants with the end of a flamed needle. With this procedure three new cultures of the organism were obtained, one as a sector from culture M and two as sectors from culture W. These were designated as M–1, W–1, and W–2, respectively.

The culture medium used for stock cultures and all experimental work, unless otherwise stated, was potato-dextrose agar (1 percent of dextrose and 1.5 percent of Bacto-agar).

Stock cultures of the black chaff organism were kept on agar slants, either at room temperature in the laboratory or in an ice box. Transfers were usually made at 2- to 4-week intervals to keep a supply of vigorously growing cultures on hand. After being kept in the laboratory for approximately 3 weeks during parts of July and August 1931, cultures M and W failed to grow when transferred to fresh agar slants, so these were no longer available for experimental work. All efforts to revive them with various culture media failed. However, cultures M–1, W–1, and W–2 did resume growth when transferred to fresh agar slants and these were used as inoculum for later pathogenicity and cultural studies.

Water suspensions of the bacteria were used as inoculum. These suspensions were made by scraping into tap water the bacterial growth from fresh colonies growing on agar slants. Usually cultures 72 to 96 hours old were used, and enough of the bacterial growth was put into the water to make a heavily clouded suspension.

When the writer began work on black chaff in the fall of 1929 no method of inoculating the plants with the pathogen had been described which was suitable for greenhouse experiments. Therefore, one of the first problems was to develop a method of inoculation for such work. Three different methods of inoculation were used: (1) Spraying the plants thoroughly with the bacterial suspension by means of an atomizer; (2) rubbing the suspension on the leaves with the fingers; (3) forcing the inoculum into the leaf roll of young plants or into the boot of older plants with a hypodermic syringe.

Spraying plants with suspensions of the bacteria in water or nutrient broth usually gave poor results, even when the plants were kept in moist chambers for 48 hours after inoculation. This was probably due to the fact that the inoculum which remained on the surface of

the plants and even the bacteria which had entered the substomatal cavities were quickly dried out and their further advance into the host checked by the low humidity existing in the greenhouse in cold weather. On the other hand, it was sometimes desirable to place these plants immediately after inoculation, without incubation in moist chambers, under the conditions in which they were to be grown. In such cases no infection resulted from the spraying method of inoculation. Likewise, when the inoculum was rubbed on the leaves with the fingers the results were either negative or not consistent enough to be reliable.

The most successful method found was that of introducing the inoculum into the leaf roll by means of a very small needle on a hypodermic syringe. The injections were made by sticking the point of the needle into, but only partially through, the leaf roll at an angle, the beveled side of the point being held toward the plant. In this way one or more injections could be made with little injury to the plant. At each injection several punctures were usually made through one or more of the rolled leaves, thus injuring several surfaces for the bacteria to attack. Whether seedlings or older plants were used, a quantity of inoculum could be forced between the rolled leaves and even out of the top of the spiral. Thus the leaf roll served as a moist chamber, protecting the inoculum from desiccation. Hereafter this method will be referred to as "hypodermic inoculation." It was the method used for all experimental inoculations unless otherwise stated.

ETIOLOGY

DESCRIPTION OF CAUSAL ORGANISM

Black chaff of wheat is caused by *Bacterium translucens* var. *undulosum* Smith, Jones, and Reddy. This organism is a monotrichous rod; hence the genus corresponds to Migula's *Pseudomonas* and the Society of American Bacteriologists' *Phytomonas*. Smith, Jones, and Reddy (*20*) did not give in detail the physiologic and cultural characteristics for this new variety but stated that in general they were the same as those described for *Bact. translucens*.[8] The only exception noted was that on whey agar the slime produced by the wheat pathogen was a deeper yellow and less fluid than that produced by the barley pathogen. This difference might not have held if they had made comparative studies with a number of isolations of the two organisms. The writer found wide variations in the color and viscosity of the bacterial growth among the five cultures (M, W, M–1, W–1, and W–2) within the variety *Bact. translucens undulosum*. On agar slants culture M was slightly deeper yellow and produced less fluid slime than culture W. However, M–1 was very light yellow and produced little slime. Cultures W–1 and W–2 were very similar to their parent culture, W, except for a slightly deeper yellow color. Since the writer found that color of colonies and fluidity of slime are not constant characters with *Bact. translucens* var. *undulosum*, he does not consider these characters a good basis for differentiating between *Bact. translucens* and *Bact. translucens* var. *undulosum*.

Two other differences between the two organisms were described by Godkin (*6*). He found that within the colonies of both bacteria

[8] JOHNSTON, C. O. DISEASES OF WHEAT IN PENNSYLVANIA AND KANSAS. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 13: 86. 1929. [Mimeographed.]

there were minute undulations or striations, as had been previously described for *Bacterium translucens* var. *undulosum* (*20*), but that they were more pronounced and appeared sooner in colonies of *Bact. translucens* var. *undulosum* than in colonies of *Bact. translucens*. The writer was unable to get authentic cultures of *Bact. translucens* to make comparisons with *Bact. translucens* var. *undulosum* in this respect.

The other difference which Godkin found between the two organisms was in their action on lactose. He stated that, in beef bouillon plus lactose, *Bacterium translucens* var. *undulosum* produced an acid reaction while *Bact. translucens* produced an alkaline reaction. These results are only in partial agreement with the results obtained by the writer. When grown on beef bouillon plus lactose, culture W and cultures W–1 and W–2, which arose as sectors from culture W, either produced an acid reaction or caused no change in the reaction of the medium. In two out of three trials culture W caused no change of reaction in the medium, while in the third trial it produced an acid reaction. No change of reaction was evident in the single trial with W–1 and W–2. In all the trials with sugars, from 6 to 10 tubes of the medium were inoculated in each case, and 4 tubes were left sterile as checks. On beef bouillon plus lactose, cultures M and M–1 produced an alkaline reaction or no change. In two out of three cases there was no change in the reaction caused by M, but in one trial with M and in the single trial made with M–1 there was a definite change to alkalinity. To the writer, these conflicting results indicate that *Bact. translucens* var. *undulosum* does not cause a specific reaction when grown on beef bouillon plus lactose, but rather that certain isolations of the organism may produce acid while others produce alkali. It is not strange that isolations of this organism should differ in this characteristic, because, as will be shown later, they differ in other respects. Since neither Jones, Johnson, and Reddy (*14*) nor Smith, Jones, and Reddy (*20*) recorded a difference in action on lactose between the two organisms, it seems likely that in this respect the cultures with which they worked were very similar to each other but different from the cultures used by Godkin.

RELATION OF ORGANISM TO HOST TISSUE

The writer was unable to find any report of work having been done to determine how the pathogen enters the host or what part of the tissues it attacks. Millasseau (*16*) made the statement that the bacteria are within the tissues of the host in older lesions, but he did not say whether they were intercellular or intracellular. Jones, Johnson, and Reddy (*14*) found that infection by *Bacterium translucens* was stomatal and that the bacteria invaded the intercellular spaces of the parenchyma in the early development of lesions. Instances of advanced invasion were found where the cell walls had partially disappeared. Their results are very similar to those obtained by the writer with *Bact. translucens* var. *undulosum*.

Bacterium translucens var. *undulosum* enters the host plant by way of the stomata and through wounds. Fresh leaves from inoculated wheat seedlings were dipped for a few minutes in a staining solution made up of 0.1 percent of thionine in 50-percent alcohol. The seedlings had been inoculated by spraying them with a water suspension of the bacteria and had been kept in moist chambers either 24 or 48

hours before being used. Examination of these leaves with the low power of a microscope showed masses of deeply stained material just beneath many of the stomata. When razor sections were cut from the infected areas and examinations made with higher magnification, these masses were found to be bacteria. Some of the bacteria had evidently got through the stomata and multiplied in the substomatal cavities. Many bacteria were found on the surface of the epidermis of the sprayed leaves, but there was no sign of entrance and multiplication except beneath the stomata. However, where punctures had been made masses of bacteria were found around the margin of the punctures, showing that they may also attack the host tissues in places where the epidermis is broken.

In order to find out what part of the tissues was attacked older lesions were embedded in paraffin and sectioned. The sections were stained with thionine and orange G. In these lesions the bacteria were both intercellular and intracellular. There were masses of bacteria in many of the parenchyma cells, but the vessels appeared to be free from invasion. In the infected areas the intercellular spaces were filled with bacteria and part of the cells were crowded out of shape. Some of the cell walls were completely broken down and the cell contents replaced with masses of bacteria, but bacteria were observed also in certain other cells the walls of which apparently were not greatly injured.

SYMPTOMS OF THE DISEASE

The most conspicuous signs of the disease appear on the glumes and awns, but Smith (*19*) has already given a good description of this phase of the disease. However, his description of the lesions on the leaves, culms, and rachises was too brief and indefinite to be of much use in recognizing the disease. Therefore, the symptoms on these parts are given.

LEAVES

The first evidence of the disease on the leaves is very small, water-soaked, dark-green spots which may be on the midrib or on any part of the leaf blade. On plants that have been artificially inoculated by forcing the inoculum into the leaf roll with a hypodermic needle, the infection usually appears first at the margins of the punctures made by the needle. On plants that have been inoculated by spraying inoculum on the leaves or by rubbing it on with the fingers and keeping the plants in moist chambers for 24 hours, the infection spots result from stomatal infections. These infection spots enlarge with the progress of the pathogen through the host tissues, mostly longitudinally, thus giving the older lesions an irregularly streaked appearance. The leaf veins usually check partially or entirely the lateral advance of the pathogen. The lesions may be limited by two adjacent leaf veins or they may involve the entire width of the leaf, in which cases those parts of the leaf beyond the infected areas soon wilt and die. The infection may progress only a millimeter or two lengthwise of the leaf, or it may extend out to the tip of the leaf or down to the ligule and even down the leaf sheath. The infected tissues gradually lose their water-soaked dark-green color and become more transparent and chlorotic. Older lesions often become dark yellow or brown and may be streaked with black. Entire lesions

sometimes become black. Under moist conditions exudate appears on the fresh lesions and may dry down into a thin scale or into small hard yellowish beads.

CULMS AND RACHISES

Light brown to black sunken streaks appear on the rachises and on the necks of the culms for some distance below the spikes. These may be narrow and scattered around the culm or they may coalesce and darken the whole circumference of the stem. Similar lesions may appear just below the nodes, especially the lower nodes, of the culms. These infected areas near the nodes appear as discolored bands and may be either entire or made up of many narrow streak lesions.

Apparently the appearance of the disease may vary on different varieties of wheat. Some varieties, especially Hope and H–44, are frequently attacked at or near the lower nodes of the culms without showing the disease on the heads and necks. In other cases, infection on these varieties may be general throughout the length of the culm. Other varieties are infected much more commonly on the necks and floral parts without any nodal infection.

GREENHOUSE AND LABORATORY EXPERIMENTS

INFLUENCE OF ENVIRONMENT ON DEVELOPMENT OF THE ORGANISM IN CULTURE

Temperature

As a preliminary step to the determination of the temperature at which the black chaff organism was most virulent, it was thought desirable to find the cardinal temperatures for its growth on culture media. Petri dishes 100 mm in diameter, containing 20 cc of agar, were inoculated in the center from a fresh transfer of culture M. Care was taken to get as nearly as possible the same amount of inoculum on each agar plate. Ten plates were then put at each of several temperatures from 0° to 40° C. The relative growths at the different temperatures were measured by taking the mean diameters of the colonies on three different dates. A few contaminated plates were discarded, but in no case were there fewer than eight colonies for measurement at any temperature. The experiment was repeated within a few days, and the average diameters of the colonies grown at the different temperatures are given in table 1.

TABLE 1.—*Effect of temperature on growth of Bacterium translucens var. undulosum on potato-dextrose agar*

Temperature (° C.)	Colonies	Average diameter of colonies in—			Temperature (° C.)	Colonies	Average diameter of colonies in—		
		2 days	4 days	7 days			2 days	4 days	7 days
	Number	*Mm*	*Mm*	*Mm*		*Number*	*Mm*	*Mm*	*Mm*
0	19	0.00	0.00	0.00	25	17	1.80	4.74	7.65
10	18	.00	.10	.44	30	19	2.19	5.65	8.74
15	17	.00	.63	1.62	35	18	.48	1.02	.98
20	18	.67	3.39	6.03	40	20	(1)	(1)	-------

[1] Very slight growth on only 3 plates.

From the results given in table 1 it is evident that *Bacterium translucens* var. *undulosum* grows best in culture at a high temper-

ature, probably slightly below 30° C., and also that it grows rapidly at temperatures ranging from 20° to 30°. The minimum temperature at which it will grow is somewhat less than 10° and the maximum approximately 40°.

Very small colonies appeared on some of the plates kept at 40° C. and remained for a few days, but by the seventh day the agar in the dishes had dried out so much at this high temperature that the colonies also had almost entirely dried up.

Humidity

The fact that black chaff is more prevalent and that the pathogen progresses through the tissues of the host more rapidly in wet weather than in dry suggested the possibility that humidity might be one of the controlling factors in the development of the disease. Experiments were made to determine the effect of different degrees of relative humidity of the surrounding atmosphere on the growth of the organism on culture media. Fresh agar slants were inoculated by touching the end of a flamed needle to a 48-hour-old colony of W–2 and then touching the adhering bacteria to the agar. Five tubes were kept at each of several constant relative humidities between 10 and 100 percent. The constant humidities were maintained by the use of aqueous solutions of sulphuric acid of known specific gravity. The different concentrations of acid for the maintenance of relative humidity of different percentages were made up according to the table given by Stevens (*21*). Seventy-five cubic centimeters of each concentration of acid were put in the bottom of rectangular glass dishes, approximately 9 by 19 cm and 6 cm deep. The inoculated test tubes were then put in the dishes above the acid; glass slides on Syracuse dishes were used to keep the tubes out of the acid. The loose cotton plugs were removed from three of the five tubes before the covers were sealed on the dishes with vaseline. The cultures were grown at laboratory temperature (approximately 21° to 25° C.). The writer realizes that the bacteria themselves were not exposed to the relative humidity maintained in the surrounding atmosphere and that the effects on growth may have been a function of the rate of drying. Since there was no noticeable difference in rate of growth between the colonies in the tubes with and without the loose cotton plugs, no differentiation is made in table 2.

Table 2.—*Effect of humidity on the growth of Bacterium translucens var. undulosum in culture*

Relative humidity (percent)	Relative growth [1] at—			Relative humidity (percent)	Relative growth [1] at—			Relative humidity (percent)	Relative growth [1] at—		
	Laboratory temperature [2]	22° C.	27° C.		Laboratory temperature [2]	22° C.	27° C.		Laboratory temperature [2]	22° C.	27° C.
100	5			60	5	4	5	30	2	0	0
90	5			50	4	3	4	20		0	0
80	5			40		2	4	10	1	0	0
70	5										

[1] The numbers 0 to 5 designate the relative amounts of bacterial growth at the different percentages of relative humidity.

[2] Approximately 21° to 25° C.

Since there were no differences in rate of growth on the medium at relative humidities of 60 percent or above and no cultures were held at the humidities of 40 and 20 percent, the experiment was repeated with humidities of 60, 50, 40, 30, 20, and 10 percent and constant temperatures of 22° and 27° C. Eight cultures were used in each dish in these trials, and the cotton plugs were removed from all after they had been placed in the dishes. Table 2 shows the relative amounts of growth on the agar slants at these temperatures and relative humidity percentages after 10 days.

It is evident from these results that a high relative humidity of the air surrounding the colonies is conducive to the most rapid growth of the black chaff organism on culture media. It is also evident that very low relative humidities may greatly retard or prohibit the development of colonies.

INFLUENCE OF TEMPERATURE ON INCUBATION PERIOD

The time required for the appearance of the first signs of black chaff after inoculation varied with the conditions under which the plants were kept. The usual incubation period for the disease on wheat seedlings was from 5 to 8 days when the temperature of the greenhouse was normal (about 18° to 22° C.) and the plants were kept in moist chambers for 48 hours after inoculation, but under optimum conditions for the development of the bacteria the incubation period was shortened. On at least two different occasions, when the temperature of the greenhouse was much higher during the day, well-defined lesions were present when the plants were removed from the moist chambers 48 hours after inoculation. When the plants were kept in a controlled-temperature room at 10°, the earliest signs of infection appeared 8 days after inoculation, and the average incubation period was from 15 to 20 days. At a controlled temperature of 20°, the earliest signs of infection appeared in 2 days after inoculation, and the average incubation period was from 5 to 7 days.

INFLUENCE OF ENVIRONMENT ON ADVANCE OF THE PATHOGEN AND ON SYMPTOMS

The length of the period of incubation is not the only phase in the development of the disease which is affected by environment. The rate of advance of the pathogen through the host tissues, the total amount of the host tissue finally affected, and the appearance of the older lesions are also affected by the conditions under which the plants are grown.

TEMPERATURE

A series of experiments were made in which the plants were placed at different temperatures and different light intensities immediately after inoculation. Hope and Kanred plants were used in the experiments, but, since there were no appreciable differences between these varieties in percentage of plants infected or in size and appearance of the lesions, the varieties are discussed as one. The conditions under which the plants were tested after the inoculation were as follows: (1) One series of the plants was kept in a controlled-temperature room at 10° C. and lighted by a single 1,000-watt lamp placed 6 to 8 feet away from the plants; (2) another series was kept at a controlled temperature of 20° and lighted by a single 1,000-watt

lamp placed 8 to 10 feet from the plants; (3) the remainder of the plants, immediately after inoculation, were moved to a greenhouse bench where they received all the available sunlight and where the temperature varied from approximately 20° to 30°. The amount of infection obtained in the several trials under the three sets of conditions is summarized in table 3.

TABLE 3.—*Effect of temperature and light during incubation period on infection of wheat by Bacterium translucens var. undulosum*

Environmental conditions			Plants inoculated	Plants infected		Length of lesions on seedling plants		Notes on adult plants
Place	Temperature	Lighting				Average	Maximum	
	° C.		Number	Number	Percent	Mm	Mm	
Control chambers	10	1,000-watt lamp at distance of 6 to 8 ft.	235	176	74.8	1-2	4-5	Lesions showed no black striping and no darkening.
	20	1,000-watt lamp at distance of 8 to 10 ft.	214	187	87.3	[1] 10-15	20-30	One plant showed slight pigmentation of lesions on 2 or 3 glumes after 30 days.
Greenhouse	20-30	All available sunlight.	324	264	81.4	-------	([2])	Lesions showed typical dark-brown or black striping within 14 to 21 days.

[1] Long water-soaked infected areas in 30 days.
[2] Some lesions extended entire length of leaves.

From the results obtained from these experiments the writer concluded the temperature at which the plants are grown is not one of the most important factors determining the percentage of plants that become infected. It can be readily seen from table 3 that there are no striking differences in the relative number of plants infected at 10° or 20° C. or at higher temperatures in the greenhouse. However, the size of the lesions or the amount of host tissue attacked by the pathogen within a given time was influenced by temperature. At 10° the infected areas on seedling plants were very small even after 30 days; the average lesions were only 1 to 2 mm long, and the longest only 4 to 5 mm. At 20°, on plants of the same age, there were long water-soaked infected areas in 30 days, the lesions averaging 10 to 15 mm in length, with some as long as 20 to 30 mm. In the greenhouse some of the lesions on the plants extended the entire length of the leaves.

LIGHT

Light intensity plays a role in determining the appearance of the older lesions on diseased plants. Lesions on adult plants kept in the controlled-temperature chambers at 10° and 20° C. under artificial illumination did not show the black striping typical of the lesions on plants approaching maturity in the field. One plant out of more than 100 that had been kept in the 20° room for 30 days showed a slight pigmentation of the lesions on two or three glumes. At 10° there was no darkening of the lesions on any of the plants. Almost all the

lesions on adult plants kept on the greenhouse bench after inoculation showed the typical dark-brown or black striping within 14 to 21 days.

VIABILITY OF THE BACTERIA UNDER ADVERSE CONDITIONS

Although Smith and his associates (*20*) suggested that one source of inoculum for spring infection might be bacteria found in the refuse and stubble left on infected fields, they presented no evidence that the bacteria could withstand the adverse conditions to which they would be subjected under natural conditions out of doors during the winter and early spring before host plants were available for attack. In order to find out whether the black chaff organism could survive the adversities of winter, the writer made some experiments during the winter and spring of 1932–33.

Forty-eight-hour-old colonies of culture W–2 on agar slants were put outside on the ground in an exposed place on December 15. Transfers to fresh agar slants were made from these colonies on December 30. In every case vigorous growth, which appeared to be the same as the original culture, appeared on the fresh agar slants. Later pathogenicity tests proved that the new growth was *Bacterium translucens* var. *undulosum*. During the time the cultures were exposed the temperature ranged from −27.8° to 11° C., showing that freezing temperatures do not quickly kill the bacteria, at least when on artificial media. Other cultures on agar were put under similar conditions December 15, 1932, and transfers were made at intervals up to April 18, 1933. Transfers made on that date, 124 days after exposure, grew vigorously.

In order to find out whether the bacteria could withstand adverse conditions approximating those to which they are subjected in wheat fields during the winter, the following experiment was made. Bacterial growth from 48-hour-old cultures of W–2 was transferred to a number of small Erlenmeyer flasks half filled with steamed soil, the steaming having been done in the autoclave at 15 pounds pressure for 1 hour after the soil had been put into the flasks. Some of the flasks contained dry soil and some wet. The soil inoculations were made by placing one loop of the bacterial growth into each flask with a flamed needle and shaking the flask until the bacteria were thoroughly mixed through the soil. Some of these flasks were put outside and exposed to cold and changing temperatures on December 15, and some were kept in the laboratory. At irregular intervals, beginning 2 days after inoculation, one flask each of the wet and of the dry soil was brought into the laboratory and transfers were made from them to agar slants. Transfers were also made from the flasks kept in the laboratory, by removing with a flamed needle one or two loops of the soil from each flask to the agar. Table 4 gives the results of the transfers after different lengths of exposure and shows definitely that *Bacterium translucens* var. *undulosum* may remain viable through a long period of continued exposure to low temperatures and to alternate freezing and thawing, conditions so detrimental to many micro-organisms. During the 124 days the cultures were left outside, the temperature ranged from −33.3° to 20° C.

Other soil cultures of the organism were put in a controlled-temperature room at −12° C. on January 31, 1933, and transfers were made from these at intervals until April 18. Vigorous growth was

obtained from these transfers on every date they were made. During the same period other soil cultures were kept in the laboratory, where the relative humidity was very low. Since the flasks were covered only with cotton plugs, the soil became very dry. However, bacteria remained viable in the dry soil throughout the 77-day period.

TABLE 4.—*Viability of Bacterium translucens var. undulosum in soil cultures after exposure to normal temperatures* [1] *out of doors between Dec. 15, 1932, and Apr. 18, 1933, at St. Paul, Minn., as determined by transfers to agar slants* [2]

Date of transfer to agar slant	Days exposed	Condition of soil		Date of transfer to agar slant	Days exposed	Condition of soil	
		Moist	Dry			Moist	Dry
1932				1933			
Dec. 17	2	+	+	Jan. 6	22	+	+
Dec. 20	5	+	+	Feb. 6	53	+	+
Dec. 30	15	−	−	Mar. 21	96	+	+
				Apr. 18	124	+	+

[1] Ranging from −33.3° to 20° C.
[2] Plus and minus signs denote whether or not transfers from soil cultures to agar slants grew.

These results indicate that *Bacterium translucens* var. *undulosum* is able to withstand adverse environmental conditions for considerable periods of time.

FIELD OBSERVATIONS AND EXPERIMENTS

OCCURRENCE OF THE DISEASE IN RELATION TO AGE OF HOST

Black chaff of wheat does not ordinarily appear in Minnesota until the plants are headed out. The progress of the disease was carefully observed in experimental plots at University Farm during 1930, 1931, and 1932. In an extensive field trial at Abilene, Kans., in 1919 Braun (*3*) observed as high as 10.5 percent seedling infection in a field of Kanred wheat that had been sown with seed from a field heavily infected with black chaff the year before. The writer has been unable to find any evidence of infection on wheat plants before they had reached the booting stage, except on plants that had been inoculated experimentally. Seed saved from plants heavily infected with black chaff in 1930 and 1931, was sown the following years (1931 and 1932) in plots parallel with plots sown to seed from uninfected plants. Thousands of plants were examined, but no seedling infection was found in these plots either in 1931 or in 1932. Later in the season, after the plants had headed, some black chaff developed, but there was no noticeable difference in the amount of infection in the plots from infected and noninfected seed. During the winters of 1931–32 and 1932–33 seed saved from infected plants and seed inoculated with cultures of the black chaff organism were planted in pots in the greenhouse. These pots were kept in moist chambers until the plants were 3 or 4 inches high, but no signs of black chaff infection appeared.

ENVIRONMENTAL INFLUENCE ON EPIDEMIOLOGY OF THE DISEASE

Continued periods of wet weather favor the development of an epidemic of black chaff. Salmon and Throckmorton (*18*) reported that the disease is often prevalent in Kansas in wet seasons. John-

ston [9] also reported that wet weather favored the disease. These reports are in agreement with observations made by the writer, particularly when the relative humidity is taken into consideration. In 1928 and 1929, particularly in 1928, there were fairly severe epidemics of the disease at University Farm, while during the seasons 1930, 1931, and 1932 there were unusually light infections. Since black chaff does not usually appear there until wheat is approaching maturity, only the weather conditions existing during June and July will be discussed in relation to the development of the disease. During these 2 months in 1930, 1931, and 1932 the weather was extremely hot and dry, while in 1928 and 1929 it more nearly approached normal. In each of the 3 years in which there was light infection the total precipitation was less and the mean monthly temperature was higher for June and July than in the 2 years when black chaff infection was heavy. The relative humidity throughout the maturative period probably had a greater influence on the development of an epidemic of black chaff than the total precipitation and mean temperature.

TABLE 5.—*Mean relative humidities (separately and combined) for June and July, 1928 to 1932, inclusive, at St. Paul, Minn.*

Year	Mean relative humidity											
	June				July				Seasonal average			
	7 a. m.	Noon	7 p. m.	Average	7 a. m.	Noon	7 p. m.	Average	7 a. m.	Noon	7 p. m.	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1928	75.0	60.0	52.0	62.3	81.9	60.2	54.5	65.5	78.5	60.1	52.3	63.6
1929	81.0	60.6	47.6	63.1	76.2	58.0	49.1	61.1	78.6	59.3	48.4	62.1
1930	75.9	52.4	51.9	60.1	76.0	46.2	48.0	56.7	76.0	49.3	50.0	58.4
1931	77.6	53.0	53.7	61.4	68.3	39.1	37.9	48.4	73.0	46.1	45.8	55.0
1932	70.0	50.0	48.2	56.1	75.4	49.2	44.7	56.4	72.7	49.6	52.3	58.2

Table 5 gives the percentage of the mean relative humidity at three different hours of the day for June and July from 1928 to 1932. Apparently there is an association of high average relative humidity with heavy black chaff infection. This association is more evident if only the 2 years 1928 and 1931 are compared. The records show that at University Farm the heaviest infections in the 5-year period were in 1928 and the lightest in 1931. During June there was only a slight difference in the average relative humidity in the 2 years, but in July there were differences of 13.6, 21.1, and 16.6 percent in the 7 a. m., 12 m., and 7 p. m. readings, respectively. The close association between high relative humidity and heavy infection and lower relative humidity and less infection indicates that the existing relative humidity does determine to some extent the amount of the disease that may develop in a given season.

GRAINS AND GRASSES AS HOSTS

Jones, Johnson, and Reddy (*13*) state that the organism causing bacterial blight of wheat was pathogenic on wheat, barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and spelt (*Triticum spelta* L.).

[9] JOHNSTON, C. O. See footnote 8.

No wider host range has since been reported, as far as can be ascertained, except that given earlier by the writer (*1*).

The writer has also obtained typical infection on einkorn (*Triticum monococcum* L.) in the field by hypodermic inoculation and on Victory oats (*Avena sativa* L.), both in the greenhouse on seedlings and in the field on adult plants. A number of other varieties of oats were inoculated in the field, but typical translucent striations developed only on Victory and Kanota. Some of the other varieties appeared to be infected soon after inoculation, but as the plants grew older the lesions became less distinct. Other workers have inoculated oats with *Bacterium translucens* var. *undulosum* but obtained no infection (*13*). Their failure to get infection might have been due to the fact that either the varieties of oats they used were resistant to *Bact. translucens* var. *undulosum* or the strain of the organism used as inoculum was not pathogenic on oats.

Wild grasses may also become infected with *Bacterium translucens* var. *undulosum*. The following species of grasses were hypodermically inoculated with culture M: *Bromus inermis* Leyss., *B. tectorum* L., *Hordeum jubatum* L., *Setaria lutescens* (Weigel) F. T. Hubb., *Agropyron repens* (L.) Beauv., *Dactylis glomerata* L., *Alopecurus geniculatus* L., and *Phalaris arundinacea* L. On *B. inermis* and *H. jubatum* irregular water-soaked striations appeared in 8 to 12 days. These became darker after a few days and were very similar to the lesions on the leaves of wheat. The other grasses did not become infected or, if infected, the lesions were not typical for black chaff on wheat.

VARIETAL SUSCEPTIBILITY IN WHEAT

All varieties of wheat are apparently more or less susceptible to the disease. At least a trace of infection can be found on field plots of most varieties of wheat in years in which there is an abundance of black chaff. Every variety tested by experimental inoculations has been susceptible to some degree. Goulden and Neatby (*7*) found in 1928 that practically every variety and strain in the nursery at Winnipeg, Canada, was attacked in some degree. At University Farm (Minnesota), the relative susceptibility of some of the more common varieties grown in the field and naturally inoculated was as follows: Hope, H–44, and Kota, very susceptible; Marquillo and Ceres, moderately susceptible; Marquis, Mindum, and Kubanka, resistant. Under other environmental conditions this comparative classification might not hold. Burton (*4, Rept. 1931*) reported that in a comparative yield test of wheat varieties carried out in 1931 at the Njaro Plant Breeding Station, Kenya, all the plants of every variety used were destroyed by black chaff, the grain being so shriveled that it could not be threshed. This report tends to bear out the conclusion of the writer that resistance to the disease is only relative.

Fifty varieties and hybrid selections were hypodermically inoculated in the booting stage with four different cultures of pathogenic bacteria. The differences noted in the virulence of the different strains of the bacteria on the varieties of wheat are indicated in the following way: 0 represents absence of the disease, and 1 to 4 represent the relative degrees of infection in any particular plot as compared with that in other plots. Table 6 gives the comparative reaction of 20 of the varieties to the four pathogens.

TABLE 6.—*Susceptibility of varieties of wheat to three cultures (W–1, W–2, and M–1) of Bacterium translucens var. undulosum from wheat and to one culture (B–1) from barley*

Variety or nursery no.	Minn. no.	C. I.[1] no.	Relative susceptibility[2] to—				Variety or nursery no.	Minn. no.	C. I.[1] no.	Relative susceptibility[2] to—			
			W–1	W–2	M–1	B–1				W–1	W–2	M–1	B–1
Marquis	1239	3641	3	3	2	3	Mindum	470	5296	3	2	3	2
Marquillo	2202	6887	2	1	1	2	Kubanka	2310	1440	3	3	1	3
Ceres	2223	6900	3	3	1	3	Minor	2612		3	4	3	
Kota	2151	5878	3	3	3	3	Komar	2244	8004	2	3	4	1
Hope	2297	8178	3	3	1	3	II–21–7	2302	10002	3	3	1	3
H–44	2301	8177	3	2	2	2	II–21–28	2303	10003	2	3	0	3
Reward	2204	8182	2	2	1	2	II–21–48	2315	10020	3	3	1	2
Reliance	2308	7370	4	4	1	3	II–21–84	2316	10021	4	2	1	3
Supreme	2309	8026	3	3	2	2	II–21–86	2607	11485	3	2	3	2
Haynes Bluestem	169	2874	3	3	2	3	II–21–94	2340	11487	3	2	1	3

[1] Accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.
[2] The numbers 0 to 4 designate the relative susceptibility of the varieties, 0 indicating absence of the disease and 4 the presence of large infected areas.

These results indicate that certain varieties may be rather resistant to some strains of the black chaff organism but quite susceptible to others. They also show that varieties like Marquis, Mindum, and Kubanka, which appear resistant in the field, may become rather heavily infected when hypodermically inoculated. The exact cause of the change in reaction to the pathogen in certain varieties is not known, but there is at least one possible explanation. Resistance to black chaff under natural conditions may be morphological or functional, as it apparently is in some cases of resistance of wheats to stem rust (*9*), and this resistance may be broken down when hypodermic inoculations are made.

COMPARISON OF PATHOGENICITY OF BACTERIUM TRANSLUCENS VAR. UNDULOSUM WITH THAT OF ISOLATIONS FROM NATURALLY INFECTED BARLEY AND RYE

In 1931 a number of varieties and selfed lines of rye at University Farm were heavily infected with a bacterial disease with symptoms closely resembling those described by Reddy, Godkin, and Johnson (*17*) for bacterial blight of rye (*Bacterium translucens* var. *secalis*). In the same year Manchuria barley was also found to be infected with a bacterial disease similar to that described by Jones, Johnson, and Reddy (*14*) as bacterial blight (*Bact. translucens*). Repeated isolations from the infected rye and barley gave yellow, slime-producing bacteria agreeing with the description of *Bact. translucens*. Marquis and Marquillo wheat inoculated with the cultures isolated from barley readily became infected. The type of lesion produced on these hosts was indistinguishable from that produced by the wheat organism. Hope wheat inoculated with cultures isolated from the infected rye was as severely attacked as when inoculated with *Bact. translucens* var. *undulosum*.

In 1932 small focal infections appeared on the first or second leaves of seedlings of several varieties and hybrid selections of barley. Cultures of bacteria isolated from these lesions were used to inoculate wheat and barley, and the same varieties were inoculated with

Bacterium translucens var. *undulosum* from wheat. In these tests the organism from barley was more virulent on barley than on wheat, and the organism from wheat was more virulent on wheat than on barley. However, from the results reported in table 6, in which the pathogenicity of one of the cultures (B–1) of the organism from barley was compared with that of cultures W–1, W–2, and M–1 of *Bact. translucens* var. *undulosum* from wheat on 20 varieties of wheat, it seems that the organism from barley is about as virulent on wheat as cultures W–1 and W–2 from wheat, and in most cases more virulent than culture M–1 from wheat, at least when experimentally inoculated into these wheat varieties.

DISCUSSION AND CONCLUSIONS

Bacterium translucens var. *undulosum* apparently is a group name including a number of strains of bacteria pathogenic to wheat. These strains may differ from each other in one or more of the following characters: (1) Color of colonies, (2) amount and fluidity of slime produced, (3) action on sugars, and (4) pathogenicity. Even among the limited number of isolates with which the writer worked, considerable differences were found with respect to the first three characters listed. These differences appeared to be as great as or greater than the differences heretofore used to distinguish the three similar organisms, *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. translucens* var. *secalis*. If there are variations within a single so-called variety as great as or greater than the differences used to distinguish the variety from other varieties, it would appear that the validity of this variety is questionable. Since such variations in cultural reactions do occur, the only basis left for distinguishing the variety is difference in pathogenicity. The question might well be raised as to how great these differences actually are.

Reddy, Godkin, and Johnson (*17*) summarized the pathogenic capabilities of the three organisms as follows:

Bacterium translucens infects only barley.
Bact. translucens var. *undulosum* infects wheat, barley, rye, and spelt.
Bact. translucens var. *secalis* infects only rye.

However, the writer found that isolates from barley and rye readily infected wheat and that the cultures of *Bacterium translucens* var. *undulosum* infected varieties of wheat, barley, rye, oats, and certain wild grasses. If it is assumed that the cultures obtained from barley and rye were *Bact. translucens* and *Bact. translucens* var. *secalis*, respectively, then no sharp difference in pathogenicity of the organisms exists. The objection may be raised, however, that the infection found on barley and rye could have been caused by *Bact. translucens* var. *undulosum*, and it would have been expected that the isolates would infect wheat. Since pathogenicity is apparently the only valid criterion for distinguishing the three organisms, it is impossible to prove that the isolates were not the wheat organism. On the other hand, if these did belong to the variety *undulosum* wide variations evidently exist within this variety. A large number of varieties of barley and rye were infected in the plots from which the isolates were obtained. If the variety *undulosum* can be found so frequently on barley and rye, then it would be difficult to determine, without making extensive cross-inoculation tests, which

organism is involved when infection is found on these hosts. But this is evidently very impracticable, and, since a number of different pathogenic strains do exist in the variety *undulosum* and probably in the other varieties of the organism too, the most logical solution to the problem of the identity of the organisms causing bacterial blight of barley and rye and black chaff of wheat would seem to the writer to be to include all the strains under a single name. He does not believe that the differences in cultural characters are great enough or consistent enough to be used as a basis for separation, nor that the pathogenic capabilities are specific enough to warrant the separation of the group into three different varieties.

The inconsistency of the present nomenclature of this group of pathogens is evident to anyone who has worked with them. Obviously, *Bacterium translucens* should be given a varietal name if it is to be used, as it is now, to designate a variety comparable with the varieties *undulosum* and *secalis*. But within *Bact. translucens* var. *undulosum* there are evidently pathogenic strains capable of infecting a greater or less number of varieties of wheat, barley, rye, oats, and wild grasses. A similar condition may be found among the strains that make up the varieties that are supposed to be restricted to barley and rye. More work must be done to determine the extent of specialization within the entire group. It seems to the writer that it would be best to use the name *Bact. translucens* for all the strains, recognizing that it is a group species comprising a number of pathogenic strains more or less specialized as to host range. Whether the strains could properly be grouped into varieties seems questionable at present.

The symptoms of black chaff have been shown to vary with different varieties and different environmental conditions. Although the disease is usually characterized by the dark striate or streak lesions on the glumes, awns, and upper parts of the culms, it may attack all parts of the plants. Goulden and Neatby (7) called attention to two different types of bacterial disease on different varieties in the wheat nursery at Winnipeg, Canada, in 1928, and suggested that there was a possibility of two organisms being responsible. In 1931, the writer examined a number of specimens of Marquillo and Ceres wheat collected at Hallock, Minn. These were infected with a bacterial disease, the appearance of which was not typical of that usually associated with black chaff. The infected areas were somewhat oily in appearance, making the surfaces seem glossy or polished. Black chaff lesions are usually dull brown or black. There are several possible explanations of the differences noted in the appearance of the disease: (1) Different strains of *Bacterium translucens* var. *undulosum* may be responsible; (2) bacteria of entirely different species may cause a similar disease; or (3) variations in environment may cause different symptoms on different hosts. Much more extensive work must be done before the exact cause or causes of the differences in symptoms can be given.

Just how and where the black chaff organism overwinters have not been definitely shown. Seed transmission evidently plays a part in the dissemination of the disease in the soft red winter wheat area. From the results of experiments the writer concludes that the pathogen may overwinter in Minnesota in the soil or on diseased stubble and refuse. Since the organism withstood long periods of subfreezing

temperatures and also remained viable after being subjected to temperatures as low as −33.3° C., it seems unlikely that low temperatures would prevent its overwintering in wheat fields in this area. Also, since the changing temperature and alternate freezing and thawing during the winter and spring did not destroy the viability of the organism in soil cultures, these conditions are probably not lethal to the organism in the field. Therefore, it seems very probable that *Bacterium translucens* var. *undulosum* does withstand the adversities of winter in wheat fields and is present to infect grain the following year. Seed transmission evidently plays a smaller part in carrying the disease over from one year to the next in the hard red spring wheat area than in some other regions, so the presence of overwintered bacteria in the soil may be one of the major factors in the incidence of the disease.

Environment limits to a great extent the development of the organism and the occurrence of the disease. In general, the same conditions favor both the growth of the organism and the development of epidemics. The best growth of the organism in culture occurs at relatively high temperatures and high relative humidities, and the most severe epidemics of the disease occur in warm, wet seasons. However, low relative humidity is probably more often a limiting factor in the development of the disease than is low temperature. Apparently, low temperatures merely retard the advance of the organism through the host tissues during cool weather without preventing its rapid development again as soon as the temperature rises. As a result the disease may advance rapidly if the days are warm, even though the nights are cool. On the other hand, the advance of the organism through the tissues already attacked may be checked for some time by the effect of low humidity drying out the lesions. The tissues of cereal plants are not bulky and are protected from desiccation only by the cuticle and the action of the guard cells in the closing of the stomata. In the infected areas the guard cells are inactivated by the attack of the pathogen and the tissues in the lesions are exposed to desiccation. Cells adjacent to those already parasitized probably are also dried out and killed, thus preventing further extension of the lesions even when more humid conditions exist later. Hence, it seems reasonable to conclude that short periods of low relative humidity have a more lasting detrimental effect on the development of the disease than periods of low temperature. As a result, one might reason that seasons in which the temperature was comparatively low and the relative humidity high would be more favorable for the appearance of an epidemic of black chaff than seasons with high temperatures and low relative humidity.

SUMMARY

Black chaff of wheat, caused by *Bacterium translucens* var. *undulosum*, appears to be very widely distributed. It occurs in the United States, Canada, and Mexico, in North America, and in Russia, France, and Belgium, in Europe. It also is found in Asia and Africa.

All aboveground parts of susceptible plants may be attacked: Leaves, glumes, awns, rachises, and culms. Some varieties are often attacked at the lower nodes of the culms, while in others the infection is usually limited to the heads and the necks of the culms.

The channels of entrance into the host are through the stomata and wounds, and the attacks are intercellular and intracellular.

The optimum temperature for the growth of the organism in culture is between 25° and 30° C., the maximum approximately 40°, and the minimum slightly below 10°.

Low relative humidity of the surrounding atmosphere retarded the growth of the organism in culture. The retarding effect was noticeable at a relative humidity of 50 percent and was greater as the percentage was lowered.

Temperature had a marked effect on the length of incubation period required. At 20° C. from 2 to 7 days were necessary for incubation, while at 10° from 8 to 20 days were required.

Low temperatures also limited the size of the lesions caused by *Bacterium translucens* var. *undulosum*. In 30 days the organism caused only very small lesions, 1 to 5 mm in length, at 10° C., while at 20° it caused much larger lesions, i. e., 10 to 30 mm in length.

The organism is resistant to extremely low temperatures, remaining viable outside in soil cultures for at least 124 days after December 15.

The disease usually does not appear in Minnesota until wheat is headed out and approaching maturity. Seedling infection rarely occurs there.

The host range of *Bacterium translucens* var. *undulosum* was found to include oats, einkorn, *Hordeum jubatum*, and *Bromus inermis* in addition to the previously described hosts—wheat, barley, rye, and spelt.

There is considerable variation in susceptibility of varieties of wheat to black chaff. Field observations over a number of years indicate that Hope, H–44, and Kota are very susceptible; Marquillo and Ceres moderately susceptible; and Marquis, Mindum, and Kubanka resistant.

Strains of the organism exist which differ in cultural and physiological characters and in pathogenicity.

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THREE BLUE-STAINING FUNGI, INCLUDING TWO NEW SPECIES, ASSOCIATED WITH BARK BEETLES[1]

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INTRODUCTION

In a previous paper[4] the writer described the association of the blue-stain fungus, *Ceratostomella pini* Münch, with the bark beetle, *Dendroctonus frontalis* Zimm., on the Atlantic coast and with *D. brevicomis* Lec. on the Pacific coast, as well as the association on the Atlantic coast of *C. ips* Rumbold with *Ips calligraphus* Germ., *I. grandicollis* Eichh., *I. avulsus* Eichh., and *I. pini* Say. Since the publication of the description of *C. ips*, in 1931, collections have been made of the fungus in all the Gulf States except Texas, and in Tennessee, Ohio, Wisconsin, and Minnesota. In the East, *C. ips* has been isolated from *Pinus banksiana* Lamb., *P. caribaea* Mor., and *P. taeda* L., in addition to many of the pines listed in the previously mentioned paper. It has also been isolated from adult *I. calligraphus*, *I. grandicollis*, and *I. avulsus*. The work here reported reveals the association in the West of *C. ips* with the bark beetles *I. oregoni* Eichh., *I. emarginatus* Lec., infesting *P. ponderosa* Dougl., and *I. integer* Eichh., infesting *P. ponderosa* and *Larix occidentalis* Nutt.

Two species of *Ceratostomella*, hitherto not described, were found, one associated with the beetle *Dendroctonus pseudotsugae* Hopk., which kills Douglas fir and infests western larch, and the other with *D. piceaperda* Hopk., which infests spruce in eastern Canada. Further it was found that the insects carried with them, besides the blue-stain fungi, two types of yeast—Zygosaccharomycetes and an anascosporogenous mycelium-forming group.[5]

METHODS

With one exception, the specimens described in this paper were collected by entomologists, who started the cultures of the fungi by dropping beetles cut out of the trees just as they were ready to emerge from their galleries or just after they had made successful attacks, into test tubes containing slanted malt agar.[6] The tubes were mailed to the laboratory, where the insects were removed. Fungi

[1] Received for publication Oct. 1, 1935; issued April 1936. The investigations on which this article was based were conducted cooperatively by the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, and the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] The writer wishes to express thanks to the members of the Division of Forest Insects, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for their collaboration, and to R. E. Balch, Division of Forest Insects, Entomological Branch, Department of Agriculture, Canada, for his cooperation.

[3] In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

[4] RUMBOLD, C. T. TWO BLUE-STAINING FUNGI ASSOCIATED WITH BARK-BEETLE INFESTATION OF PINES. Jour. Agr. Research 43: 847-873, illus. 1931.

[5] A study of these organisms is being made by Eugene C. Holst, student assistant, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[6] RUMBOLD, C. T. See footnote 4.

appearing regularly in the tubes were assumed to have started from spores on or in the bodies of the beetles. Cultures were also obtained in the laboratory by transferring frass, slivers of the gallery walls, or adjacent stained wood to malt agar. Cultures from the wood were compared with the cultures obtained from the beetles. A few larvae and eggs cut out of the galleries also were used. The entomologists examined the material to make sure that insects of other species than the one reported were not present.

Except when otherwise stated, the material used for description and measurement was taken from cultures grown on malt agar. The cultures were kept where the temperature ranged from 11° to 30° C. with a usual temperature of 24°.

CERATOSTOMELLA IPS

The blue-stain fungus, *Ceratostomella ips*, has already been described,[7] but certain additional facts about the species in general are given and certain necessary corrections are made in the present paper. For the western strains, listed in table 1, only the descriptions and measurements that differ from those of the eastern and Japanese strains are given.

TABLE 1.—*Source of western strains of Ceratostomella ips*

Date collected	Locality	Species of tree	Species of beetle	Infestation period (days)	Remarks
July 1930	Northport, Wash.	*Pinus ponderosa.*	*Ips oregoni.*	Unknown.	Tree killed in recent fire. Blue-stained wood used as inocula.
September 1930.	Klamath Falls, Oreg.	do	do	14	Inocula were interior blue-stained wood.
	Sisters, Oreg.	do	*I. integer.*	2	Inocula were tissues from gallery wall and frass.
	do	do	do	21	Inocula were tissues from gallery walls.
	do	*Larix occidentalis.*	do	21	Green windfall. Inocula were tissues of entrance and gallery walls and interior stained wood.
June 1932	do	*Pinus ponderosa.*	*I. emarginatus.*	14	Standing tree with fading needles. Inocula were gallery walls.
	do	do	do	21	Cultures started from larval mines cut from green windfall.
	Coeur d'Alene, Idaho	do	*I. oregoni.*	About 65	Inocula were emerging adult beetles cut from standing tree.
	Sisters, Oreg.	do	*I. integer.*	About 24	Inocula were adult beetles cut from a green windfall.
	do	do	do	21	Green windfall. Perithecia in the galleries. Gallery walls used as inocula.
June 1933	do	do	*I. emarginatus.*	Unknown.	Adult beetles cut from standing tree used as inocula.
	Coeur d'Alene, Idaho	do	*I. oregoni.*	About 7	Do.

THE FUNGUS IN IPS-INFESTED BARK AND WOOD

The appearance of *Ceratostomella ips* in infested bark and wood in the West is typical of that found in the East. Additional information obtained in the West showed that the perithecia of *C. ips* lined

[7] RUMBOLD, C. T. See footnote 4.

the galleries, their bases being buried in the gallery walls. When the galleries were occupied by the bark beetles, they were clear; neither the mycelium nor perithecia of a blue-stain fungus could be seen. The insects apparently cut off the protruding perithecial necks to keep the passages clear. Perhaps they used this material as food. A number of perithecia were found in other specimens showing crooked necks, with nodes where the necks had apparently grown out after being pruned. After the beetles had emerged the abandoned galleries were often filled with the protruding bristlelike necks of the perithecia. Leach, Orr, and Christensen [8] found ascospores and fragments of perithecia of *C. ips* in exoskeletons and scattered in the intestinal tracts of *Ips pini* and *I. grandicollis*. They also found viable ascospores in the frass of the beetles.

THE FUNGUS IN CULTURE

Yeasts were the first organisms to develop in the original cultures started from the bodies of insects. As the test-tube cultures aged, the blue-stain fungus appeared as white hyphae growing out from the yeast colonies; the fungus frequently grew over the yeasts and covered the entire surface of the slant. The manner of growth, the color, and the general appearance of the mycelium, conidia, and conidiophores (figs. 1 and 2) resemble the eastern strains of *Ceratostomella ips*.

Single-spore cultures [9] were used in the study of the western strains of *Ceratostomella ips*. These were selected after they had developed normal perithecia and conidiophores. The majority of single-spore cultures failed to grow and fruit normally, and therefore confirmed the results obtained by Leach [10] when studying this fungus. Cultures from beetles resembled those isolated from infested bark and wood.

Table 2 gives a summary of the mean dimensions of perithecia of *Ceratostomella ips* isolated from beetles of three western species of *Ips* and, for comparison, the grand mean dimensions of the strains collected in the Atlantic States. In general the size of the base of the western strains varies no more than that of the eastern strains. The mean measurements of the bases of the eastern and western strains are approximately the same. The mean length of neck is shorter in the western strains. It has been noticed that the length of the neck is much more variable than the size of the base.

Figure 3, *A* and *B*, shows outline drawings of typical perithecia of the strains of *Ceratostomella ips* associated with the eastern and western species of *Ips*.

[8] LEACH, J. G., ORR, L. W., and CHRISTENSEN, C. THE INTERRELATIONSHIPS OF BARK BEETLES AND BLUE-STAINING FUNGI IN FELLED NORWAY PINE TIMBER. Jour. Agr. Research 49: 315–341, illus. 1934.

[9] Made by L. J. Meuli, formerly scientific assistant in the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[10] LEACH, J. G. THE PRODUCTION OF PERITHECIA IN CERATOSTOMELLA IPS RUMB. Phytopathology 24: 1037–1040. 1934.

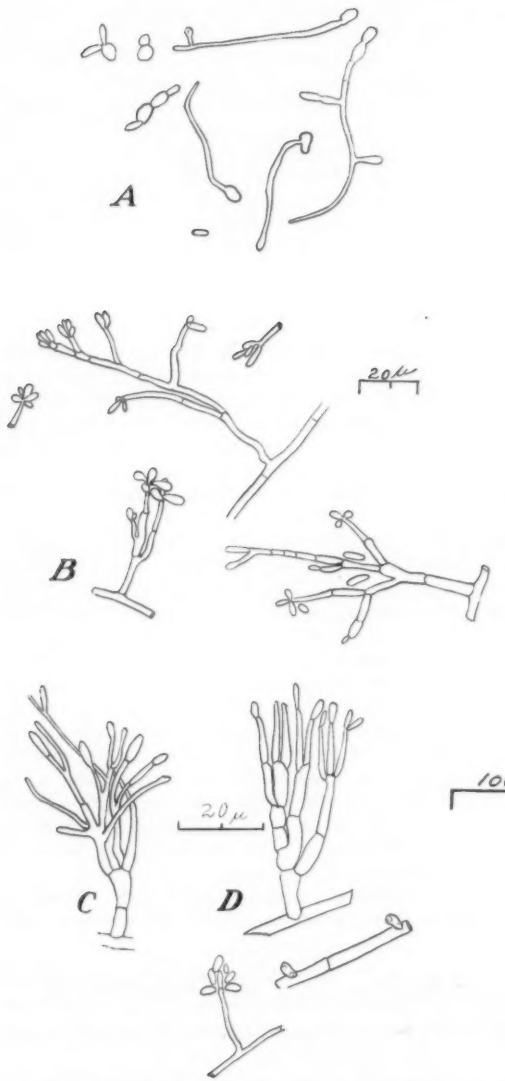

FIGURE 1.—Camera-lucida drawings of *Ceratostomella ips* growing in Van Tieghem cells on malt agar. *A* and *B*, Strain isolated from adult *Ips integer* collected in Oregon: *A*, Germinating conidia in a 1-day-old culture; *B*, conidia and conidiophores when culture was 3 days old; *C*, strain isolated from adult *I. oregoni* collected in Idaho; conidia and conidiophores in a 2-day-old culture; *D*, strain isolated from adult *I. emarginatus* collected in Oregon; mycelium, conidia, and conidiophores in a 2-week-old culture.

FIGURE 2.—A fascicle of mature conidiophores of *Ceratostomella ips* associated with *Ips integer*.

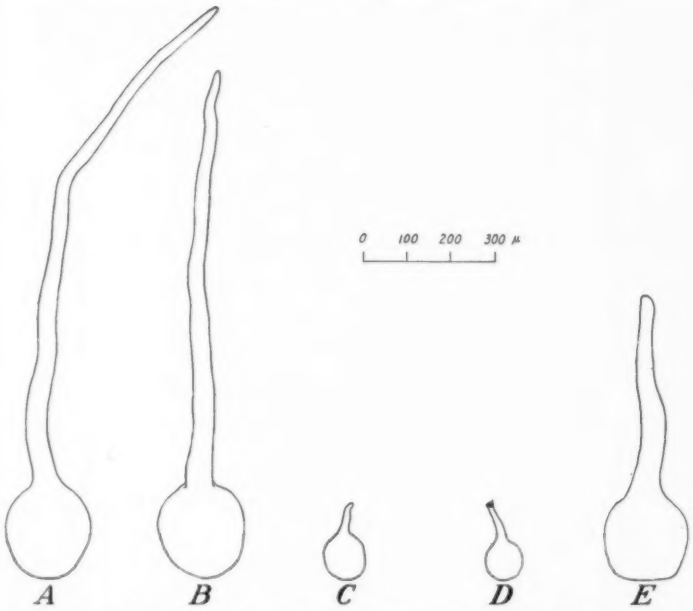

FIGURE 3.—Outlines of perithecia drawn according to average dimensions: *A, Ceratostomella ips* associated with eastern species of *Ips; B, C. ips* associated with western species of *Ips; C, C. pini* associated with some eastern and western species of *Dendroctonus; D, C. pseudotsugae* associated with *D. pseudotsugae; E, C. piceaperda* associated with *D. piceaperda*.

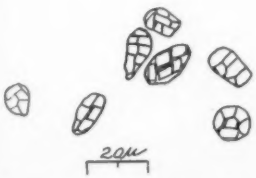

FIGURE 4.—Asci of a strain of *Ceratostomella ips* associated with *Ips emarginatus*.

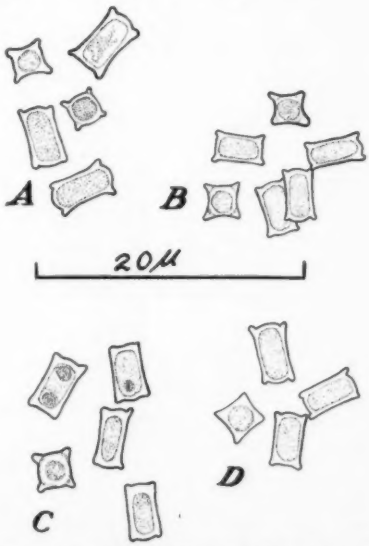

FIGURE 5.—Ascospores of *Ceratostomella ips* associated with (*A*) *Ips emarginatus*, (*B*) *I. oregoni*, (*C*) *I. integer*, and (*D*) *I. calligraphus*.

TABLE 2.—*Summary of mean dimensions of perithecia of Ceratostomella ips from eastern and western strains grown on malt agar*

HEIGHT OF BASE

Associated insect	Source		Strains of *Ceratostomella ips* having perithecia with a mean [1] dimension of—								Grand mean	Perithecia of *Ceratostomella ips* having a mean dimension of—							
	Host	Region	150μ	170μ	190μ	210μ	230μ	250μ	270μ	290μ		150μ	170μ	190μ	210μ	230μ	250μ	270μ	290μ
			Number	Number	Number	Number	Number	Number	Number	Number	μ	Number	Number	Number	Number	Number	Number	Number	Number
Ips spp.	2- to 3-needled pines	Atlantic States [3]	1	1	3	7	2				206	19	67	74	284	70			
I. integer	*Pinus ponderosa*	Oregon			6	5	1	1			198			227	99	10	7		
Do	*Larix occidentalis*	do			1						192			50					
I. emarginatus	*P. ponderosa*	do		1			1	1	1	1	236		52			3	47	31	20
I. oregoni	do	Oregon, Washington, Idaho		2	1	1					191		52	50	22				
Total												19	171	401	405	83	54	31	20

WIDTH OF BASE

Ips spp.	2- to 3-needled pines	Atlantic States [3]	1	3	2	8					198	19	110	95	290				
I. integer	*P. ponderosa*	Oregon			8	4	1				197			249	87	7			
Do	*L. occidentalis*	do			1						195			50					
I. emarginatus	*P. ponderosa*	do			1		1	1	1	1	238			52		3	47	31	20
I. oregoni	do	Oregon, Washington, Idaho		2	1	1					187		52	50	22				
Total												19	162	496	399	10	47	31	20

LENGTH OF NECK

			Strains of *Ceratostomella ips* having perithecia with a mean dimension of—								Grand mean	Perithecia of *Ceratostomella ips* having a mean dimension of—							
			600μ	800μ	1,000μ	1,200μ	1,400μ	1,600μ	1,800μ	2,000μ		600μ	800μ	1,000μ	1,200μ	1,400μ	1,600μ	1,800μ	2,000μ
			Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	μ	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Ips spp.	2- to 3-needled pines	Atlantic States [2]	1	2	2	2	3	2	2	------	1,273	19	81	51	87	159	61	56	------
I. integer	*P. ponderosa*	Oregon	------	9	4	------	------	------	------	------	849	------	255	88	------	------	------	------	------
Do	*L. occidentalis*	------do	1	------	------	------	------	------	------	------	664	50	------	------	------	------	------	------	------
I. emarginatus	*P. ponderosa*	------do	1	1	3	------	------	------	------	------	968	3	52	98	------	------	------	------	------
I. oregoni	------do	Oregon, Washington, Idaho	1	1	------	1	1	------	------	------	1,209	29	23	------	50	22	------	------	------
Total			------	------	------	------	------	------	------	------	------	101	411	237	137	181	61	56	0

[1] The round numbers (microns) of the column headings are the midpoints of the groups into which the actual means fall.
[2] Previously reported in more detail (see footnote 4).

The asci (fig. 4) resemble those found in the eastern strain but differ from the Japanese strain in that the ascospores fill the asci much more than is reported by Nisikado and Yamauti.[11]

When pressed out of the perithecia the ascospores of eastern and western strains have the shape of quadrangular prisms (fig. 5), not cylinders as was stated in the technical description of the eastern strains of this fungus in 1931.[12] The shape of the ascospores may be seen more readily if stained with picro-aniline blue. So treated, the walls of the spores become yellow and the interior blue. The edges of the walls of the quadrangular-prism-shaped spores look like flanges, while the blue-stained interiors are ellipsoidal or cylindrical. Figure 5, *A*, *B*, and *C*, shows the side and end views of spores of three western strains of *C. ips*, and, for comparison, spores of an eastern strain, isolated from a specimen of *Ips calligraphus*, are shown in *D*.

CERATOSTOMELLA PSEUDOTSUGAE N. SP.

Ceratostomella pseudotsugae n. sp. was collected during three summers on specimens of the species listed in table 3.

THE FUNGUS IN BARK AND WOOD INFESTED WITH DENDROCTONUS PSEUDOTSUGAE

The Douglas fir beetles, *Dendroctonus pseudotsugae*, bore through the outer bark to the phloem or to the outer layer of wood and there extend their galleries. *Ceratostomella pseudotsugae* grows from these galleries, usually in longitudinal streaks (fig. 6). The fungus covers the inner bark and wood with a black mycelial growth, which is usually mixed with particles of dried rosin. The stain of the inner bark caused by this fungus is not so noticeable as that caused by *C. pini*, possibly because the numerous black sclerotia so characteristic of the latter are not present. The fungus grows in toward the heartwood, staining the sapwood gull gray to slate gray.[13] The medullary rays become black.

TABLE 3.—*Source of Ceratostomella pseudotsugae n. sp. associated with Dendroctonus pseudotsugae*

Date collected	Locality	Species of tree	Infestation period	Remarks
			Days	
September 1931	Metaline Falls, Wash.	*Pseudotsuga taxifolia.*	39	Specimen showed living larvae. Sporulating perithecia growing in gallery walls.
	do	do	38	Do.
	do	do	38	Specimen showed living beetles and larvae. Conidiophores in the larval mines.
	do	do	(1)	Attack by beetles began in May 1931. Adult beetles cut out of bark as they were emerging. 4 larvae cut out of their mines. 23 of the 24 cultures started developed perithecia.
June 1932	do	do	4	Inner bark tissues from entrance chamber used as inocula.
	do	do	10	Eggs cut from galleries used as inocula.
	do	*Larix occidentalis.*	7	Large tree felled May 20, 1932. Frass used as inocula.
	Sisters, Oreg	*Pseudotsuga taxifolia.*	14	A green windfall of large size. Bark tissue from gallery used as inocula.
June 1933	Metaline Falls, Wash.	do	(1)	Beetles cut from bark as they were emerging. 10 out of 11 cultures started developed perithecia.

[1] Unknown.

[11] NISIKADO, Y., and YAMAUTI, K. CONTRIBUTIONS TO THE KNOWLEDGE OF THE SAP STAINS OF WOOD IN JAPAN. I. STUDIES ON CERATOSTOMELLA IPS RUMBOLD, THE CAUSE OF A BLUE STAIN OF PINE TREES IN WESTERN JAPAN. Ber. Ōhara Inst. Landw. Forsch. 5: 501-538, illus. 1933.

[12] RUMBOLD, C. T. See footnote 4.

[13] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

FIGURE 6.—Inner bark of *Pseudotsuga taxifolia* (LaMarck) Britton showing galleries of *Dendroctonus pseudotsugae*. Blue stain caused by *Ceratostomella pseudotsugae* extends from the galleries. The tiny black dots are perithecia.

On the black mycelial growth are tiny perithecia (fig. 3, *D*) with short curved necks. The combination of beetle galleries and spreading fungus loosens the bark. In a short time ants, mites, nematodes, and various molds are found under the bark.

Ceratostomella pseudotsugae first invades the ray-parenchyma cells of the wood and later the tracheids, passing from cell to cell through the pits.

THE FUNGUS IN CULTURE

Cultures of *Ceratostomella pseudotsugae* were started from adult beetles, from slivers of the insect gallery walls, and from frass, eggs, and larvae. All these cultures developed yeast at first, and later the blue-stain fungus grew out from the yeast colonies, often covering them completely.

In culture this fungus develops a large amount of aerial mycelium and produces a lighter-colored mycelium, more conidia, and fewer perithecia than those developed in *Ceratostomella pini* associated with *Dendroctonus brevicomis* and *D. frontalis*. In this respect it resembles somewhat a strain of *C. pini* found in the East on *Pinus virginiana* Mill., unassociated with *Dendroctonus*,[14] and a strain found on the Pacific coast, isolated from a lightning-injured ponderosa pine. However, the lack of sclerotia, both when grown on malt agar and on pine, distinguishes this fungus from *C. pini*, and it differs also in its physiological reactions.

Ceratostomella pseudotsugae and three strains of *C. pini* were grown on media having different sources of nitrogen.[15] To the standard basal medium (containing 2.0 percent of glucose, 0.50 percent of acid potassium phosphate, 0.1 percent of magnesium sulphate, 0.0016 percent of bromcresol purple, and 1.5 percent of Bacto-agar) was added 0.5 percent of one of the following nitrogenous compounds: Urea, asparagine, sodium caseinate, or peptone. The media were all adjusted to approximately pH 6.2. Table 4 gives the reactions. By observing the reaction changes of the media over a period of time, or the presence or absence of growth and the type of growth, it was found that the three strains of *C. pini* reacted alike, growing abnormally and producing acid when supplied with sodium caseinate or peptone but not growing on asparagine or urea; *C. pseudotsugae* grew well on all the media, producing a basic reaction on those containing sodium caseinate or peptone. These physiological reactions emphasize the distinction between *C. pseudotsugae* and *C. pini*.

TABLE 4.—*Reaction of Ceratostomella pini and C. pseudotsugae on media containing different nitrogenous compounds*

Culture and source	Reaction to indicated compound after specified period					
	Sodium caseinate			Peptone	Asparagine	Urea
	3 days	8 days	28 days	21 days	21 days	21 days
Ceratostomella pini no. 187a from *Dendroctonus frontalis*, North Carolina.	Acid	Acid	Acid	Acid	No growth.	No growth.
C. pini no. 416 from *D. brevicomis*, Oregon.	do	do	do	do	do	Do.
C. pini no. 421 from uninfested wood, Oregon.	do	do	do	do	do	Do.
C. pseudotsugae no. 436 from *D. pseudotsugae*, Washington.	do	Neutral.	Basic	Basic	Growth	Growth.

[14] RUMBOLD, C. T. See footnote 4.
[15] These tests were made by Eugene C. Holst.

When the fungi are grown on autoclaved pine, the difference between a strain of *Ceratostomella pini* isolated from an adult *Dendroctonus brevicomis* and the fungus isolated from a *D. pseudotsugae* beetle is easily seen. *C. pini* covers the surface of the wood quickly with a black stain consisting of innumerable perithecia, black sclerotia, and dark-colored mycelium. The wood has a black granular appearance. *C. pseudotsugae* stains the wood a gull gray, the aerial mycelium is hyaline to gray, but most of the surface discoloration is caused by the numerous perithecia.

TABLE 5.—*Dimensions of perithecia of Ceratostomella pseudotsugae from Dendroctonus pseudotsugae beetles or wood infested with them*

GROWN ON MALT AGAR

Source and strain number of *Ceratostomella pseudotsugae*	Fruits measured	Height of base			Width of base			Length of neck		
		Extreme range	Sextile range[1]	Mean	Extreme range	Sextile range[1]	Mean	Extreme range	Sextile range[1]	Mean
	Number	μ	μ	μ	μ	μ	μ	μ	μ	μ
From *Pseudotsuga taxifolia*, 1931:										
No. 383	26	60-120	80-110	95	60-120	80-110	94	40-120	60- 90	74
No. 384	91	50-130	70-100	85	50-120	70-100	85	40-110	55- 90	75
From *Larix occidentalis*, 1932:										
No. 423b	50	70-120	80-100	90	60-130	80-110	91	30-140	50- 80	67
From *P. taxifolia*, 1932:										
No. 423a	30	45-140	70- 90	81	45-140	70-100	84	20-100	60- 90	76
No. 423c	46	50-130	80-110	90	50-120	80-110	89	30-110	50- 75	60
Associated with *Dendroctonus pseudotsugae*, 1932:										
No. 423:										
Adult 1	63	60-102	65- 80	73	55-100	64- 80	74	45-125	73-110	93
Adult 2	20	55- 90	60- 80	70	55- 83	65- 75	70	40-100	40- 63	54
Adult 3	31	55-100	70- 93	82	50-110	70- 90	79	55-160	78-130	103
Adult 9	36	61-100	70- 95	82	42-110	70- 90	81	30-130	90-115	103
Adults 7, 10, 11, 12, 13, 17	33	50-130	60- 90	80	50-120	60- 90	80	45-120	60-100	80
Associated with *D. pseudotsugae*, 1933:										
No. 436:										
Adults 3, 5, and 7	24	60-120	75-110	91	50-100	70-100	80	30- 90	40- 80	62
Adult 6	30	60-130	70- 90	80	60-130	65- 80	77	45-130	65-100	82
Adults 8 and 10	31	60-110	70-100	85	60-110	70-100	81	40- 90	50- 80	67
Adult 9	74	50-140	70-110	87	50-130	70-110	87	45-130	60-110	90
Total, range, or mean	585	45-140	70-100	84	42-140	70-100	83	20-160	55-100	79

GROWN ON WOOD

Associated with *D. pseudotsugae* in *P. taxifolia*:										
No. 435, adult 6, grown on *Pinus taeda*	40	45-100	60- 80	70	40-100	60- 80	70	30-120	50- 95	74
No. 383, original perithecia growing on inner bark of *Pseudotsuga taxifolia*	13	50- 90	50- 80	69	50- 80	60- 80	68	35-110	45- 90	70

[1] The sextile range discards the sixth of the measurements at each end of the extreme range, permitting an approximation of the frequency distribution.

A mature culture of *Ceratostomella pseudotsugae* contains white gray, and snuff-brown mycelium at its top, and numerous small, often incompletely developed perithecia. Black perithecia embedded in a warm sepia and black mycelium, surrounded and sometimes covered by gray aerial mycelium, appear in the center of the slant. The individual perithecia cannot be seen easily with the naked eye but are visible with a hand lens. At the base of the slant, wedged

between the glass and agar, a black sclerotoid layer of mycelium sometimes forms. The fungus grows readily but not vigorously on steam-sterilized southern yellow pine. A culture, made in the forest from gallery-wall tissue, when 10 days old contained gray, brown, and black mycelium and mature perithecia, but transplants from this strain remained light-colored and failed to produce spores. The perithecia developed on wood are somewhat smaller than those growing on malt agar (table 5).

Mycelium

The young mycelium growing in culture forms a white mat covering the surface of the agar. The young hyaline hyphae are septate, branch irregularly, and measure 1.2μ to 4μ in diameter. They form strands, anastomose, and change color gradually. The hyphae turn brown with age and the cell divisions become numerous, so that old hyphae often consist of short cells that may become globular. The walls thicken, and often a dark granular exudation appears. Old dark-colored hyphae may be relatively thick, varying from 4μ to 15μ in diameter. The cells forming the old hyphae often become polyhedral and in this respect resemble those forming the sclerotia of *Ceratostomella pini*. However, their walls are not so thick nor so firm as sclerotial cells.

Conidia and Conidiophores

At room temperature (about 25° C.) the first conidia appear about 48 hours after the germination of ascospores. They grow directly from the hyphae at first and are obovoid. Later they form in clusters at the tips of simple hyaline conidiophores. As the conidiophore lengthens, the conidium is pushed to the side of the growing tip. The conidiophores remain small, are usually unbranched (fig. 7, *A*), and do not change color as the culture ages. The conidia vary in shape from globular or obovoid to clavate, and in size from 2.7μ to 5μ by 1.4μ to 2.7μ.

Perithecia

Beneath the bark of the tree the black perithecia develop on the dark mycelium, which spreads from the galleries between the inner bark and the wood. The fruits generally are flattened by the pressure of the bark. In this situation the base of the perithecium is bare of hyphae except those attaching it to the substratum. In culture perithecia are found with basal hyphae growing into the air, looking like bristles. They are straight, septate, brown, tapering at the tip, about as long as the width of the base of the perithecium. The ostiole at the end of a short curved neck is surrounded by short hyaline cilia from 10μ to 23μ long (fig. 3, *D*). Ten have been counted at an ostiole. An ostiole through which ascospores have been ejected is generally torn. The globular perithecial base is dark brown and the tip of the neck light brown.

The perithecia somewhat resemble those of *Ceratostomella pini* (fig. 3, *C*). The measurements of the perithecia are given in table 5.

Asci

So far only immature asci have been seen. These were clavate and later polyhedral, owing possibly to their being crowded in the interior of the base. The eight spores are irregularly arranged. The walls of the asci disappear before the ascospores are ejected. There

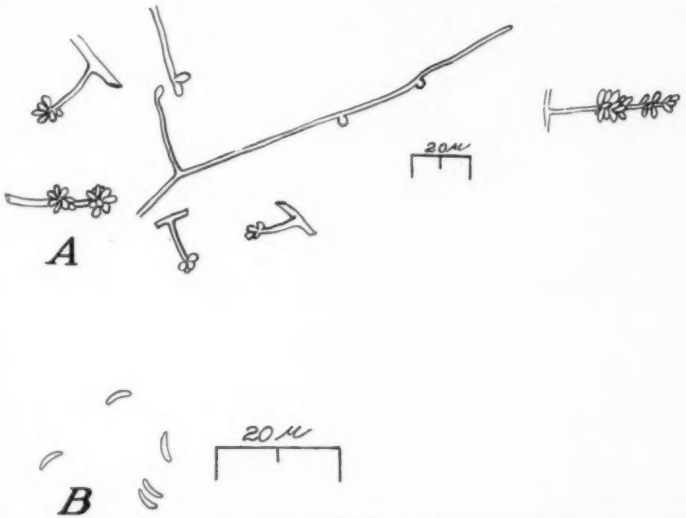

FIGURE 7.—*Ceratostomella pseudotsugae* growing in Van Tieghem cell cultures 1 to 5 days old: *A*, Conidia and conidiophores; *B*, ascospores.

is an oily substance formed with the spores that often is ejected before the spores.

Ascospores

The ascospores are crescent-shaped, slender, and pointed at both ends (fig. 7, *B*). They are hyaline, with a thin mucilaginous coating which causes them to clump together when ejected on the tip of the neck of the perithecium. The glistening clumps of ascospores are conspicuous. The mean length of the ascospores is 4μ, and the mean width 1.5μ.

TECHNICAL DESCRIPTION

Ceratostomella pseudotsugae n. sp.

Young colonies with conidia white changing to sepia and black, with perithecia; young hyphae hyaline, 1.2μ to 4.0μ in diameter; old hyphae brown, 4μ to 15μ in diameter; conidia hyaline, appearing first on hyphae, globular, obovoid to clavate, ranging from 2.7μ to 5μ by 1.4μ to 2.7μ; conidiophores single, hyaline, usually unbranched, hyphae bearing conidia in clusters; conidia solitary, adherent, forming a head; perithecia black, globose, slightly hirsute; height of base, extreme range 45μ to 140μ, sextile range 70μ to 100μ, mean 84μ; width of base, extreme range 42μ to 140μ, sextile range 70μ to 100μ, mean 83μ; length of neck, extreme range 20μ to 160μ, sextile range 55μ to 100μ, mean 79μ; diameter of neck near base 25μ, at ostiole 10μ, the ostiole with hyaline terminal cilia 10μ to 23μ long;

asci ephemeral, polyhedral; ascospores 8, hyaline, crescent-shaped; length, extreme range 2.4μ to 5.4μ, sextile range 3.5μ to 4.8μ, mean 4μ; width, extreme range 0.9μ to 2.4μ, sextile range 1.2μ to 1.9μ, mean width 1.5μ.

On sapwood of *Pseudotsuga taxifolia* infested with *Dendroctonus pseudotsugae* near Metaline Falls, Wash., and near Sisters, Oreg.; and on *Larix occidentalis* infested with *D. pseudotsugae*, near Metaline Falls, Wash.

DIAGNOSIS

Ceratostomella pseudotsugae sp. nov.

Coloniis juvenilibus conidiferis albis, tum brunneis-nigrescentibus cum perithecciis; hyphis hyalinis, 1.2μ–4.0μ diam., dein brunnescentibus, 4μ–15μ diam.; conidiophoris simplicibus, hyphis plerumque sine ramis conidia globosa ferentibus; conidiis hyalinis, in hyphis primo apparentibus, globosis, obovoideis vel clavatis, solitariis deinde in massulas congregatis, 2.7μ–5.0μ usque 1.4μ–2.7μ; perithecciis nigris, globosis leniter hirsutis, 45μ–140μ imo altis, 42μ–140μ latis; rostellis 20μ–160μ longis, 10μ–25μ diam., ostiolo filamentis hyalinis, terminalibus, 10μ–23μ longis, praedito; ascis evanidis, polyhedricis, octosporis; ascosporis hyalinis, lunatis, 2.4μ–5.4μ longis, 0.9μ–2.4μ latis.

In ligno sapido *Pseudotsugae taxifoliae* et *Laricis occidentalis Dendroctono pseudotsugae* infestato, Washington et Oregon, U. S. A.

CERATOSTOMELLA PICEAPERDA N. SP.

Ceratostomella piceaperda n. sp. was found on the following specimens collected in the localities named:

Picea glauca (Moench) Voss, infested 15 days with *Dendroctonus piceaperda*, collected in the forest near St. Peters, Cape Breton, Nova Scotia, Canada, in June 1930.

Picea glauca, infested 1 month with *Dendroctonus piceaperda*, collected in the forest near Grand Cascapedia, Bonaventure County, on the Gaspé Peninsula, Province of Quebec, Canada, in July 1931.

An adult beetle of *D. piceaperda*, in July 1933, cut out of the bark of *P. glauca*. The tree stood in a forest near St. Peters, Cape Breton, Nova Scotia, Canada, and had been recently attacked.

The blue-stain fungus isolated from these specimens resembles in many respects a German fungus, *Ceratostomella penicillata* Grosmann,[16] isolated from *Picea excelsa* Link infested with *Ips typographus* L. and *Pityogenes chalcographus* L. The measurements of the perithecium of the German fungus are: Diameter of base, 250μ to 300μ; length of neck, 300μ to 500μ. The ascospores measure 6.5μ by 2.3μ. The height of conidiophores varies from 450μ to 600μ, diameter 7μ to 8μ, conidia 11μ to 12μ by 3μ to 3.5μ. The conidia and conidiophores of the Canadian fungus are smaller. The conspicuous conidiophores of the German strain also differ from the Canadian in the shape and size of their heads.

THE FUNGUS IN BARK AND WOOD INFESTED WITH DENDROCTONUS PICEAPERDA

Dendroctonus piceaperda will invade living spruce trees, although it prefers the bark of dying windfalls and fresh stumps. It bores through the outer bark to the phloem or to the outer layer of wood and there extends its egg galleries (fig. 8). *Ceratostomella piceaperda* has been found fruiting in these galleries and growing from them, commonly in longitudinal streaks. A close examination of the blackened areas showed that the inner bark and cambium were covered with a network of dark-brown hyphae. Perithecia were seen only in the galleries.

[16] GROSMANN, H. ÜBER DIE SYSTEMATISCHEN BEZIEHUNGEN DER GATTUNG LEPTOGRAPHIUM LAGERBERG ET MELIN ZUR GATTUNG CERATOSTOMELLA SACC. NEBST EINIGEN BEMERKUNGEN ÜBER SCOPULARIA VENUSTA PREUSS UND HANTZACHIA PHYCOMYCES AWD. Hedwigia 72: 183–194, illus. 1932.

Spruce stained with *Ceratostomella piceaperda* is gull gray in color. The fungus grows first in the rays, turning them black, then invades the tracheids by means of the bordered pits. In culture the surface of infected wood appears furry because of the numerous perithecia and conidiophores covering it.

THE FUNGUS IN CULTURE

Cultures of *Ceratostomella piceaperda* were developed by transferring slivers of blue-stained inner bark to malt agar. All the plantings produced yeast at first; later the blue-stain fungus appeared and grew over the yeast. The fungus was cultured with difficulty, the warm summer temperature at the laboratory (up to 30° C.) apparently being unfavorable for its growth. The cultures started from specimens collected at Grand Cascapedia in 1931 did not develop perithecia until the spring of 1932 and died during that summer. The cultures, started from a beetle collected in July 1933, began to develop perithecia 5 months later. A strain collected in 1930 has been in cultivation in the laboratory for 4 years. It is usually kept at 11° to 25°. The fungus developed mature perithecia in 14 days when growing on steam-sterilized pine (*Pinus caribaea*). It hydrolyzes boiled rice, producing a liquor. If it is combined with a yeast which also was isolated from *Dendroctonus piceaperda* infested bark, the hydrolyzation of the rice is stimulated, and a liquid is produced

FIGURE 8.—Bark of *Picea glauca* infested 15 days with *Dendroctonus piceaperda*. Blue stain caused by *Ceratostomella piceaperda* starting to spread from galleries.

that has a fruity aroma. The yeast alone barely grows on the rice. The presence of this yeast in a malt-agar culture markedly stimulates the growth and fruiting of the fungus.

A mature culture of *Ceratostomella piceaperda* grown on a malt agar slant is chaetura black. The aerial mycelium is usually hair brown at the top of the slant and black at the base. The surface of the culture has a velvety appearance due to the growth of conidiophores. These conidiophores become dark brown as they age and surround the perithecia, whose necks point up like shiny black thorns from the black substratum. Perithecia and conidiophores will be found developing ascospores and conidia at the same time, the fungus being unlike *C. pini* and *C. ips* in this respect.

Mycelium

The cultures used in the description of this fungus originated from a single conidium. The young mycelium is white, then cream-colored. The young hyphae, which measure 2.6μ to 5.5μ in diameter, form loose strands and change color quickly. A culture 4 days old, grown at 25° C., is dark brown in the center. The hyphae walls thicken, and, as the culture ages, hyphae may be found having a diameter of 17μ.

Conidia and Conidiophores

At 25° C. the first conidia appear on the hyphae 24 hours after the germination of a conidium. The conidia forming on the hyphae are hyaline, and obovoid or clavate (fig. 9, *A*). When germinating, the conidium swells and often becomes globular. Some conidia bud like yeast. On others from 1 to 4 germination tubes have been seen. Simple short hyaline conidiophores appear that develop clavate conidia on their tips, but when 2 days old the fungus develops the characteristic conidiophores that resemble *Leptographium lundbergii* Lagerberg and Melin. The conidiophores are straight 5- to 7-septate brown hyphae, growing erect from the vegetative hyphae; near the tip a branch forms with hyaline subbranches, which develop the conidia at their tips. These conidia are held in a drop of a mucilaginous liquid on the top of the stalk (fig. 10). As the conidiophore ages, the color of the drop changes from white to yellow and then to brown. The conidiophores with their conidia seem to play an important part in the life cycle of this fungus, if vigor of growth is a criterion.

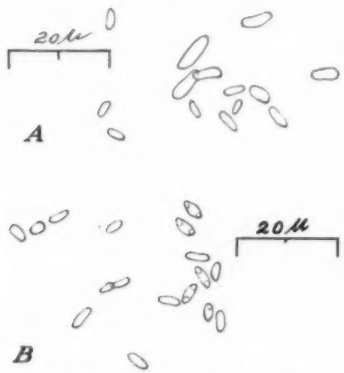

FIGURE 9.—*Ceratostomella piceaperda*: *A*, Conidia; *B*, ascospores.

Perithecia

Beneath the bark of the spruce the perithecia develop in the insect galleries, their bases embedded in the bark tissues. In this situation the base is bare of hyphae. In culture the globular base develops

many-septate brown hyphae, which grow out into the air. These basal hyphae average about 50μ in length. The ostiole at the tip of the neck is without cilia (fig. 3, *E*). To the eye the perithecium is

FIGURE 10.—Conidiophores of *Ceratostomella piceaperda* from an 8-day-old culture. × 175.

jet black. Some of the perithecia show a proliferation of the neck. The sizes of the perithecia are given in table 6.

ASCI

Only immature asci have been seen. They are hyaline and rather pear-shaped. Before the ascospores are ejected, however, the walls of the asci disintegrate. There are eight ascospores in an ascus.

ASCOSPORES

The ascospores are hyaline and ellipsoid (fig. 9, *B*). They have a thin coating of a mucilaginous substance which causes the spores to clump together on the tips of the perithecial necks, where they form a glistening mass.

TABLE 6.—*Dimensions of perithecia of Ceratostomella piceaperda*

Media	Fruits measured	Height of base			Width of base			Length of neck		
		Extreme range	Sextile range [1]	Mean	Extreme range	Sextile range [1]	Mean	Extreme range	Sextile range [1]	Mean
	No.	μ	μ	μ	μ	μ	μ	μ	μ	μ
Malt agar	76	90–350	140–240	197	80–340	130–250	193	130–860	270–650	451
Rice agar	11	120–200	130–190	159	130–200	140–180	152	110–660	170–495	337
Wood	106	100–250	150–210	173	110–250	150–210	176	220–980	460–800	615
Total, range, or mean	193	90–350	150–230	181	80–340	140–220	181	110–980	360–660	535

[1] The sextile range discards the sixth of the measurements at each end of the extreme range, permitting an approximation of the frequency distribution.

TECHNICAL DESCRIPTION

Ceratostomella piceaperda n. sp.

Young conidia-producing colonies white to cream, changing to chaetura black; young hyphae hyaline, 2.6μ to 5.5μ in diameter, old hyphae brown, 6μ to 17μ in diameter; conidia hyaline, appearing first on hyphae, later on conidiophores, obovoid or clavate, extreme range 3μ to 11μ by 2μ to 4μ, mean 5.4μ by 3.2μ; conidiophores straight, brown, 5- to 7-septate, erect, branched, with conidia developing terminally on hyaline subbranches and collecting in a mucilaginous drop; conidiophores 170μ to 250μ high, stalk 4μ to 8μ in diameter; perithecia black, globose, hirsute; height of base, extreme range 90μ to 350μ, sextile range 150μ to 230μ, mean 181μ; width of base, extreme range 80μ to 340μ, sextile range 140μ to 220μ, mean 181μ; length of neck, extreme range 110μ to 980μ, sextile range 360μ to 660μ, mean 535μ; diameter of neck near base 45μ, at ostiole 25μ; ostiole without terminal cilia; asci ephemeral; ascospores 8, hyaline, ellipsoid; extreme range 3.6μ to 4. 7μ by 1.0μ to 2.4μ, sextile range 3.9μ to 4.4μ by 1.9μ to 2.0μ, mean 4.3μ by 2.0μ.

On sapwood of *Picea glauca* infested with *Dendroctonus piceaperda* near St. Peters, Nova Scotia, Canada, and near Grand Cascapedia, Quebec, Canada.

DIAGNOSIS

Ceratostomella piceaperda sp. nov.

Coloniis juvenilibus conidiferis albis cremeisve, tum brunneis-nigris cum perithecis; hyphis juvenilibus hyalinis, 2.6μ–5.5μ, senescentibus brunneis, 6μ–17μ diam.; conidiophoris erectis, brunneis, ramosis, 170μ–250μ altis, 4μ–8μ latis; conidiis hyalinis, obovoideis vel clavatis, 3μ–11μ longis, 2μ–4μ latis, in hyphis primo apparentibus, apice ramulorum hyalinorum gestis, in guttam mucosam conglobatis; peritheciis nigris, globosis, hirsutis, 90μ–350μ altis, 80μ–340μ latis; rostellis 110μ–980μ longis, 25μ–45μ diam., ostiolo carente filamentis; ascis evanidis, octosporis; ascosporis hyalinis, ellipsoideis, 3.6μ–4.7μ longis, 1.0μ–2.4μ latis.

In ligno sapido *Piceae glaucae Dendroctono piceaperda* infestato, Nova Scotia et Quebec, Canada.

SUMMARY AND CONCLUSIONS

That certain bark-boring beetles carry specific fungi with them on their flights has been established by an accumulation of data collected during a number of years.

Ceratostomella ips, which causes the blue stain of sapwood of conifers on the Atlantic coast when infested with eastern species of *Ips* and which causes a blue stain of pine trees in western Japan, has been found to be associated also with the bark beetles *Ips emarginatus*, *I. integer*, and *I. oregoni*, which infest conifers on the Pacific coast.

Technical descriptions of two new fungi associated with bark beetles are given.

Ceratostomella pseudotsugae n. sp. is associated with *Dendroctonus pseudotsugae*, which infests Douglas fir and larch on the Pacific coast.

Ceratostomella piceaperda n. sp. is associated with *Dendroctonus piceaperda*, which infests spruce in eastern Canada.

RELATION OF ACCESSORY GROWTH SUBSTANCES TO HEAVY METALS, INCLUDING MOLYBDENUM, IN THE NUTRITION OF ASPERGILLUS NIGER [1]

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INTRODUCTION

The data presented in this paper were obtained initially in an attempt to develop a method for the complete removal, by extraction with alcohol, of heavy metals from the constituents employed in a nutrient solution for *Aspergillus niger* Van Tiegh. Though the method proved inapplicable for the purpose in mind, the results were extended and amplified once their significance in investigations on the accessory growth substances required by micro-organisms was realized. Correlation of the data on the heavy-metal requirements with those on the accessory growth bodies was considered also to be of importance.

The first observation on the existence of accessory bodies essential for the nutrition of the fungi was that of Wildiers (*13*),[2] who in 1901 announced the discovery of "bios", a hypothetical organic substance that he considered essential for the nutrition of yeast. This substance he found to be present in the filtrates of old yeast cultures and in apparently pure sucrose. It could be extracted from sucrose with 80-percent alcohol. The subsequent discovery of vitamins and of their importance in animal nutrition has led to the belief that "bios" functions similarly with the fungi. Investigators, however, have not agreed upon the necessity of "bios" and similar substances for the nutrition of fungi and bacteria, although the difficulties associated with the maintenance of pure cultures of micro-organisms in artificial media are not disputed. Effect of quantity of inoculum, dying out of cultures, and alterations in virulence and other functions are phenomena recognized by all.

REVIEW OF LITERATURE

Of the voluminous literature on the accessory substances required by micro-organisms, mention may be made of a few recent papers. Hall, James, and Stuart (*5*) have reported that recrystallization of sucrose with alcohol eliminated a substance believed to be an organic nitrogen compound (bios) the presence of which is necessary for the growth of yeast. Extraction of sucrose with alcohol, on the other hand, results in the removal of another hypothetical organic substance ("coenzyme R"), not identical with bios, that is essential for the development of *Rhizobia*, according to Allison, Hoover, and Burk (*2*).

Claims of the existence of other accessory growth substances also have been made. Nielsen and Hartelius (*6*) state that *Rhizopus suinus* elaborates two such substances, one of which is identical with

[1] Received for publication Nov. 2, 1935; issued April 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 448.

heteroauxin, regarded by Went (*12*) as a growth hormone of green plants. The second substance is "growth substance B", an accessory body for growth of *Aspergillus niger*. Vitamin B, the antineuritic growth factor, is considered by Schopfer (*8*) to be essential for the development of certain of the mucors. Peskett (*7*) has published an extensive review of the literature.

METHODS

Both extraction with 95-percent alcohol and recrystallization with 80-percent alcohol have been employed for the removal of contaminants from sucrose in these investigations. The sucrose utilized was a highly refined sugar containing 0.002 to 0.0025 percent ash. Other chemicals were of reagent grade. The water was redistilled in Pyrex glassware. The organism was grown for 5 or 6 days at 34.7° to 35.1° C., in 200-cc Erlenmeyer flasks containing 50 cc of nutrient solution. The inoculum per flask averaged 0.05 mg (dry weight) of spore material.

Further details regarding the composition of the solutions used, the method of nutrient-solution purification, and other features will be found in former publications by the writer (*10*, *11*). All solutions contained 5 percent of sucrose and the necessary salts.

The values tabulated are each the average of two duplicate cultures. The percentage yields are obtained by dividing the yield in the solution with an element omitted by the yield with the element present. That is, the yield in the complete nutrient, referred to as the maximum yield, is taken as equal to 100 percent in each experiment.

GROWTH WITH SUCROSE PURIFIED BY MEANS OF ALCOHOL

The components of the nutrient solution were extracted with freshly distilled 95-percent alcohol for 6 hours or longer in a Pyrex extraction apparatus. The sucrose dissolved partially, the ammonium nitrate completely, while the dipotassium phosphate and the magnesium sulphate were practically insoluble. The ammonium nitrate was permitted to crystallize from the solvent, which was filtered off, and the crystals were dried at 103° C. to remove alcohol. The crystals of the other compounds were dried similarly. All were kept in a desiccator over anhydrous calcium chloride until required for use. The compounds retained the identical chemical formulas that they had originally except in the case of magnesium sulphate. Ignition of a sample of the latter compound after extraction with alcohol revealed that it had changed from heptahydrate to dihydrate; 0.38 g of the latter is formed from 0.6 g of the former.

Table 1 shows growth of the fungus in a nutrient solution (dibasic) composed of 1,000 cc of water redistilled in Pyrex glassware, 50 g of sucrose, 2 g of ammonium nitrate, 0.48 g of dipotassium phosphate and 0.6 g of heptahydrate (or 0.38 g of dihydrate) magnesium sulphate. One experiment is with unextracted ingredients, another with extracted sucrose but unextracted salts, and the third with components all of which had been extracted with alcohol. Iron, zinc, copper, and manganese, when added to the solution, were in amounts of 0.20, 0.14, 0.04, and 0.02 mg per liter, respectively.

TABLE 1.—*Effect of alcoholic extraction of the nutrient-solution [1] components upon the iron, zinc, copper, and manganese requirements of Aspergillus niger*

Heavy metal [2] omitted	Reagent compounds not extracted (initial solution, pH, 7.13)				Sucrose extracted (initial solution, pH, 7.05)				All components extracted (initial solution, pH, 6.99)			
	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
All	80.7	7.60	2.48	6, bl.	37.3	4.33	2.82	4, j.	29.6	3.59	3.08	2, bl.
Fe	340.5	32.09	1.89	4, bl.	291.7	33.86	1.85	4, j.	422.7	51.73	1.79	6, bl.
Zn	111.5	10.51	2.34	6, bl.	60.4	7.01	2.61	4, j.	40.3	4.93	2.74	4, j.
Cu	959.9	90.45	2.44	2, bl.	780.4	90.60	1.82	2, w.	771.9	94.46	1.91	4, y.
Mn	821.0	77.37	1.94	2, bl.	672.8	78.11	1.64	2, w.	768.7	94.09	1.76	6, bl.
None	1,061.2	100.00	2.48	6, bl.	861.4	100.00	1.75	4, j.	817.2	100.00	1.80	6, bl.

[1] The nutrient solution (dibasic) was composed of 1,000 cc of water redistilled in Pyrex glassware, 50 g of sucrose, 2 g of ammonium nitrate, 0.48 g of dipotassium phosphate, and 0.6 g of heptahydrate (or 0.38 g of dihydrate) magnesium sulphate.

[2] Heavy-metal concentrations were as follows: Fe, 0.20; Zn, 0.14; Cu, 0.04; and Mn, 0.02 mg per liter.

[3] Yield from complete medium with no metal omitted taken as 100 percent.

[4] Sporulation is indicated as 0 (sterile) to 10 (black with spores), and spore color by the initial letter or letters of the words jet, black, brown, tan, yellow, and white.

A distinct decrease in yield is shown in both cases as the result of extraction with alcohol. Not only is the yield diminished in the minus-heavy-metal and the minus-zinc cultures, but it is also lower in the full-nutrient cultures. Apparently the decrease in yield is due partially to the removal of zinc from the sucrose and the salts. Sufficient zinc is thus removed to cause a decrease in the percentage of maximum yield from 10.51 to 4.93 in the minus-zinc cultures. Additional trials with the full-nutrient solution revealed that the supply of zinc had become deficient and that the maximum yield averaged about 940 mg when the zinc removed from the sucrose (0.02 mg Zn per liter) was replaced, or approximately 13.2 percent less than with unextracted sucrose. The decrement in yield with the full-nutrient culture appeared to be dependent to some extent upon the duration of extraction, but was not due primarily to a deficiency of any of the known constituents of the nutrient solution.

Further studies were concerned, therefore, with the identification of the unknown material responsible for the diminution in maximum yield. The effects of 55 elements, including the 12 known chemical elements of the nutrient solution, were tested on the growth of *Aspergillus niger* in the nutrient solution under discussion, and in several other deficient nutrient solutions as well. The net result of these experiments here applicable may be briefly summarized by the statement that molybdenum is essential for growth and sporulation of this fungus. Its addition made up the deficiency in maximum yield resulting from the use of purified sucrose. A comparison of the results obtained with untreated sucrose, extracted sucrose, sucrose recrystallized once with 80-percent alcohol, and sucrose three times so recrystallized is given in table 2.

TABLE 2.—*Effect of alcohol purification of sucrose upon the growth and development of Aspergillus niger in relation to role of heavy metals, with special reference to molybdenum*

Heavy metal omitted [1]	Sucrose not purified (initial solution, pH 7.22)				Sucrose extracted 6 hours (initial solution, pH 7.12)				Sucrose extracted 6 hours (initial solution, pH 7.23) [2]			
	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
All	49.8	4.46	2.81	2, bl.	27.0	2.48	2.96	2, bl.	27.2	2.48	2.80	2, bl.
Fe	275.4	24.68	1.81	4, bl.	309.6	28.45	1.83	4, bl.	302.4	27.49	1.83	4, bl.
Zn	105.5	9.45	2.35	6, bl.	38.5	3.54	2.78	4, lb.	50.0	4.55	2.67	4, bl.
Cu	1,019.4	91.35	2.56	2, t.	1,059.3	93.35	2.78	2, t.	1,029.4	93.59	3.12	2, t.
Mn	934.4	83.74	1.81	4, bl.	930.6	85.51	1.88	4, bl.	814.3	74.03	1.80	2, bl.
Mo	1,018.1	91.15	1.88	4, bl.	1,004.3	92.28	1.90	6, bl.	984.3	89.49	1.93	6, bl.
None	1,115.9	100.00	2.68	8, bl.	1,088.3	100.00	2.55	8, bl.	1,099.9	100.00	2.84	6, bl.

Heavy metal omitted [1]	Sucrose extracted 12 hours (initial solution, pH 7.17)				Sucrose recrystallized (initial solution, pH 7.06)				Sucrose recrystallized 3 times (initial solution, pH ?)			
	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]	Yield	Proportion of maximum yield obtained [3]	Acidity at harvest	Sporulation [4]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
All	32.0	2.87	2.96	2, bl.	29.4	2.65	2.92	2, bl.	35.3	3.25	2.87	3, bl.
Fe	336.0	30.11	1.81	4, bl.	340.1	30.71	1.76	4, bl.	422.3	38.89	1.73	6, bl.
Zn	53.0	4.75	2.65	4, bl.	50.6	4.57	2.73	4, bl.	38.8	3.57	2.74	3, bl.
Cu	1,039.9	93.19	3.07	2, t.	997.6	90.07	2.94	2, y.	950.6	87.53	3.07	2, t.
Mn	844.7	75.70	1.82	2, bl.	956.7	86.38	1.82	2, bl.	998.5	91.94	1.85	2, bl.
Mo	881.1	78.96	1.86	4, bl.	962.2	86.87	1.84	6, bl.	1,000.4	92.12	1.94	7, bl.
None	1,115.9	100.00	2.83	6, bl.	1,107.6	100.00	2.66	8, bl.	1,086.0	100.00	2.76	7, bl.

[1] The heavy-metal concentrations used were as follows: Fe, 0.20; Zn, 0.14; Cu, 0.04; Mn, 0.02; and Mo, 0.01 mg per liter, except that in experiments 3 and 4 the zinc content was 0.16 mg per liter.
[2] The extraction apparatus was thoroughly cleaned of residues from previous extraction.
[3] See footnote 3, table 1.
[4] See footnote 4, table 1.

The effects of purification of sucrose with alcohol at low impurity levels appear to be the same whether extraction or recrystallization is employed. They differ, however, with the individual heavy metal present in the sucrose. Zinc and molybdenum are removed partially by these procedures, although the results with the latter are rather erratic. Copper is removed to a slight extent, especially through repeated recrystallization. Iron and sometimes manganese, however, give definite indications of an increase in concentration in the sucrose through this procedure. It will be observed that the maximum yields are practically identical. It should be added, however, that filtration was omitted during the recrystallization process.

The results of the experiments with purified sucrose are more intelligible if it is kept in mind that the sucrose employed is of an exceptional grade of purity, containing not over 0.0025 percent ash. With sucrose of a significantly lower degree of purity the effects of purification become far greater. It should not be forgotten, moreover, that the comparisons usually made in studies on accessory bodies would correspond to the contrasts in the present investigation between

cultures containing sufficient nutrients for maximum growth and cultures deficient in more than one constituent.

Additional data bearing upon the need of *Aspergillus niger* for molybdenum are shown in table 3. The data are from solutions differing in composition and in treatment in order to avoid so far as practicable the possibility of error through the use of a single and perhaps abnormal solution. The cultures were grown for 5 days. The first two experiments were performed simultaneously, the addition of iron sulphate (25 mg per liter) in combination with calcium carbonate being practically without effect. The added iron is removed and possibly a trace of copper added. The results in these two experiments are quite uniform and serve to illustrate the experimental precision that may be attained with duplicate cultures. The results obtained in the next experiment demonstrate that the data obtained with Pyrex glassware are practically identical with those with quartz. Such differences as exist are probably due to the use of decantation instead of filtration in the experiment with quartz. In this experiment, water redistilled in quartz and quartz vessels were employed in all operations except measurement. In the last experiment equivalent amounts of magnesium nitrate and sodium sulphate were substituted for the magnesium sulphate of the dibasic nutrient solution. Comparison of the results with those in the first experiment of table 2 indicates the presence in the magnesium sulphate of significant amounts of iron, manganese, and particularly molybdenum.

TABLE 3.—*Effects of modifications in nutrient solution composition and treatment on the growth and development of Aspergillus niger with heavy metals, particularly with molybdenum*

Heavy metal omitted	Dibasic nutrient solution purified with $CaCO_3$ (pH 7.02)				Dibasic nutrient solution purified with $CaCO_3$+$FeSO_4$ (pH 6.98)			
	Yield	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [3]	Yield	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [3]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
All	9.2	0.93	3.33	1, l.	10.8	1.06	3.16	1, l.
Fe	20.7	2.10	3.10	1, bl.	21.3	2.10	2.99	1, bl.
Zn	16.7	1.70	3.14	1, l.	22.7	2.24	3.06	1, bl.
Cu	744.3	75.58	1.87	2, t.	807.7	79.55	1.93	2, t.
Mn	771.4	78.33	1.80	2, bl.	805.9	79.38	1.80	2, bl.
Mo	901.2	91.51	1.71	4, l.	925.8	91.18	1.75	4, l.
None	984.8	100.00	2.13	4, bl.	1,015.3	100.00	2.16	4, bl.

Heavy metal omitted	Dibasic nutrient solution purified with $CaCO_3$ in quartz vessels and culture flasks (pH 7.00)				Untreated dibasic solution [1] modified by substitution of $Mg(NO_3)_2$ and Na_2SO_4 for $MgSO_4$ (pH 7.22)			
	Yield	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [3]	Yield	Proportion of maximum yield obtained [2]	Acidity at harvest	Sporulation [3]
	Mg	*Percent*	*pH*			*Percent*	*pH*	
All	7.4	0.78	3.18	1, l.	52.4	5.07	2.53	2, bl.
Fe	28.5	3.00	2.95	1, l.	174.4	16.86	2.00	4, bl.
Zn	15.0	1.58	3.00	1, l.	80.4	7.77	2.04	6, bl.
Cu	839.2	88.22	2.17	3, t.	916.9	88.66	2.51	2, w.
Mn	899.3	94.53	1.67	3, l.	754.5	72.96	1.81	2, l.
Mo	930.2	97.78	1.93	6, bl.	677.9	65.55	1.72	2, bl.
None	951.3	100.00	2.06	6, bl.	1,034.2	100.00	2.36	8, bl.

[1] Equivalent amounts of $Mg(NO_3)_2.6H_2O$ and Na_2SO_4 were substituted for $MgSO_4.7H_2O$.
[2] See footnote 3, table 1.
[3] See footnote 4, table 1.

As a final check upon the necessity of molybdenum for the development of the fungus, three other salts of this element were tested. Besides the ammonium molybdate customarily employed, a sample of sodium molybdate from the same manufacturer was also used. In addition, samples of both these salts purchased from another manufacturer were tested in a similar manner. The results obtained with all molybdenum salts were quite similar and indicated the necessity for this element in the nutrition of *Aspergillus niger*.

GROWTH WITH MISCELLANEOUS ORGANIC SUBSTANCES

The results shown in table 4 were obtained in an attempt to determine the effects of traces of organic materials stated to contain accessory growth substances or of the alleged growth substances themselves upon the growth of *Aspergillus niger*. The absence of any increase in maximum yield (compare experiment 1, table 2) as a consequence of the addition of these materials would indicate that

TABLE 4.—*Effect of miscellaneous organic substances upon the growth of Aspergillus niger in a dibasic nutrient solution*

Heavy metal[1] omitted	Heavy inoculum[2] used with solution (initial pH, 7.19)				Vitamin B[3] present in solution (initial pH, 7.18)				Vitamin C[3] present in solution (initial pH, 7.21)			
	Yield	Proportion of maximum yield obtained[6]	Acidity at harvest	Sporulation[7]	Yield	Proportion of maximum yield obtained[6]	Acidity at harvest	Sporulation[7]	Yield	Proportion of maximum yield obtained[6]	Acidity at harvest	Sporulation[7]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*pH*		*Mg*	*Percent*	*pH*	
All	45.9	4.30	2.75	6, bl.	34.2	3.43	2.88	4, bl.	30.0	2.91	2.81	4, bl.
Fe	419.1	39.27	1.78	6, bl.	154.7	15.52	2.08	4, bl.	242.3	23.47	1.88	4, bl.
Zn	87.5	8.20	2.41	6, bl.	36.2	3.63	2.81	4, bl.	51.7	5.01	2.68	4, bl.
Cu	855.3	80.14	3.56	4, t.	792.5	79.52	2.21	1, t.	910.3	88.19	2.54	1, t.
Mn	965.6	90.47	1.73	2, bl.	782.7	78.54	1.78	2, bl.	788.7	76.41	1.72	2, bl.
Mo	922.3	86.41	1.83	6, bl.	783.2	78.59	1.69	3, l.	792.2	76.75	1.68	4, l.
None	1,067.3	100.00	3.12	8, bl.	996.6	100.00	2.48	6, bl.	1,032.2	100.00	2.54	6, bl.

Heavy metal[1] omitted	Yeast decoction[4] present in solution (initial pH, 7.15)				Malt extract[5] present in solution (initial pH, 7.17)			
	Yield	Proportion of maximum yield obtained[6]	Acidity at harvest	Sporulation[7]	Yield	Proportion of maximum yield obtained[6]	Acidity at harvest	Sporulation[7]
	Mg	*Percent*	*pH*		*Mg*	*Percent*	*PH*	
All	230.4	21.88	2.16	6, bl.	212.3	20.11	2.01	6, br.
Fe	572.5	54.36	1.92	4, l.	469.8	44.41	1.84	4, l.
Zn	264.6	25.12	2.00	8, bl.	262.4	24.86	1.95	8, bl.
Cu	1,012.8	96.16	3.29	4, t.	984.1	93.22	3.28	4, y.
Mn	932.2	88.51	1.70	6, l.	952.7	90.24	1.77	6, l.
Mo	914.9	86.87	1.96	8, bl.	1,002.2	94.93	2.54	8, bl.
None	1,053.2	100.00	2.96	9, bl.	1,055.7	100.00	2.96	8, bl.

[1] Heavy-metal concentrations were as follows: Fe, 0.20; Zn, 0.16; Cu, 0.04; Mn, 0.02; and Mo, 0.01 mg per liter.
[2] Inoculum 20 times standard or about 1.0 mg per flask.
[3] Vitamin B_1 or vitamin C at 5.0 mg per liter.
[4] Yeast decoction, 1.0 cc of a 1-percent solution per liter (filtered).
[5] Malt extract 10 cc of a 1.6-percent solution per liter (filtered).
[6] See footnote 3, table 1.
[7] See footnote 4, table 1.

growth of the "W" strain of *Aspergillus niger* used in these experiments is not dependent upon accessory growth substances. Owing to the writer's inability to obtain a culture of *Rhizopus suinus* at this time, no test of Nielsen's "growth substance B" could be made. It should be noted, however, that Nielsen's maximum yield for *Aspergillus niger* is $1,168 \pm 68$ mg for 2.5 g of sucrose, while that obtained by the author with recrystallized sucrose and without accessory growth substances has been as high as 1,192.0 mg per 2.5 g of sucrose.

The data of table 4 indicate that growth increases obtained by investigators with accessory growth materials such as yeast extract, malt extract, and similar substances derived from organic materials are due, at least partially, to the presence of heavy metals in these substances. The experiments of table 4, in fact, may be considered as biological assays of the iron, zinc, copper, manganese, and molybdenum content of these materials. A crude approximation of the heavy-metal content introduced can be arrived at if proportionality in cause and effect is assumed. The difference in percentage maximum yields between the test solution and the control, divided by the similar difference between the maximum and the control, times the optimum concentration, equals the amount of heavy metal introduced.

AN OPTIMUM NUTRIENT SOLUTION

It was pointed out in a former publication (*10*) that the mineral constituents contained in the Pfeffer optimum solution and in the Raulin optimum solution were excessive in quantity because of the failure to include all heavy metals essential for the growth of *Aspergillus niger*. The optimum solution (dibasic), as determined by the writer, suffers from the same defect, since no molybdenum was added. Table 5 affords a comparison of these solutions and of a revised dibasic solution which includes molybdenum (0.02 mg per liter), as well as iron (0.20 per liter), zinc (0.18 per liter), copper (0.04 per liter), and manganese (0.02 per liter). The ammonium nitrate, dipotassium phosphate, and magnesium sulphate were used in amounts of 1.90 g, 0.35 g, and 0.25 g per liter, respectively. The yield at 35° C. is approximately 1,175 mg per 2.5 g of sucrose for a growth period of 5 days. The coefficient of utilization, therefore, is about 47 percent.

TABLE 5.—*A comparison of optimum solutions giving maximum yields of Aspergillus niger*

Solution	Total content of salts per liter	Heavy metals added	Milligrams per liter of indicated element				
			N	K	P	Mg	S
	Grams						
Pfeffer	17.50	Fe	3,500.0	1,435.0	1,140.0	247.5	325.0
Raulin	6.77	Fe, Zn	1,017.0	250.0	108.0	66.0	53.0
Dibasic	3.08	Fe, Zn, Cu, Mn	700.0	215.5	[1] 85.4	59.4	[2] 78.0
Revised dibasic [3]	2.50	Fe, Zn, Cu, Mn, Mo	665.0	157.1	[1] 61.3	24.8	[2] 32.5

[1] Phosphorus probably in excess.
[2] Sulphur in excess. Actual optimum about 20 mg per liter.
[3] Includes heavy metals in following concentrations: Mo, 0.02; Fe, 0.20; Zn, 0.18; Cu, 0.04; and Mn, 0.02 mg per liter; also NH_4NO_3, 1.90; K_2HPO_4, 0.35; and $MgSO_4.7H_2O$, 0.25 g per liter.

DISCUSSION

The evidence obtained with respect to the need of the fungus for molybdenum is quite convincing. The action of molybdenum is specific as judged by a comparison with that of salts of 54 other chemical elements, and apparently is not due to the presence of an impurity in the molybdenum compounds employed. Its presence aids both growth and sporulation and hastens development so that the maximum yield is attained a day earlier. The results, furthermore, cannot be attributed to any peculiarity of the nutrient solution, inasmuch as several other nutrient solutions gave similar results. Lastly, its inclusion in the formula of the optimum solution results in a decrease in the total quantity of salts per liter required for maximum growth from 3.08 g to 2.50 g, or about 19 percent.

The fact that growth is still appreciable even though no molybdenum has been added to the nutrient solution is without doubt due to unremoved traces of molybdenum. The spores used for inoculating the cultures also probably contain traces of this element. The same condition exists with respect to iron and zinc, and especially copper and manganese. In experiments reserved for publication in another connection it has been possible to obtain much greater differences in growth with and without addition of molybdenum to the nutrient solution. The yields are only about 10 to 12 percent of those in the complete nutrient solution, or poorer than those reported for iron, copper, and manganese in this paper.

Bortels (*3*, *4*) has claimed that molybdenum and probably vanadium are necessary for the process of nitrogen fixation in *Azotobacter* and in *Rhizobia*. Schröder (*9*), moreover, considers molybdenum, as well as iron, zinc, copper, tungsten, silica, and perhaps calcium, essential for nitrogen fixation by *Azotobacter*. Though later investigators have noted also that growth is accelerated by molybdenum, the impression remains that a special connection exists between molybdenum and nitrogen fixation. It is doubtful, however, in view of the results with *Aspergillus niger*, whether the needs of these bacteria for molybdenum are limited to the process of nitrogen fixation. Molybdenum, in all probability, aids nitrogen fixation primarily because it is an element essential for growth and development, even though it may play a specific part in nitrogen metabolism.

The successive decreases in the quantities of nitrogen, potassium, phosphorus, magnesium, and sulphur required for maximum yields, with more complete provision of the essential heavy metals,[3] probably constitute a factor in agricultural practice as well as in nutrient-solution studies. On the other hand, though the chemical elements required only in minute quantities may be assumed to be present in the majority of soils in amounts sufficient for maximum yield of green plants, it seems likely that at least some if not many exceptions occur. The determination of the chemical element essential in minute quantities for plants cannot be considered, therefore, as a problem of only abstract importance.

No evidence could be obtained that *Aspergillus niger* ("W" strain) requires the presence of accessory growth substances for its normal growth and development. The diminution in yields of this organism, obtained as a result of alcoholic purification of the sucrose of the

[3] The presence of molybdenum, as well as of iron, zinc, copper, and manganese, has been detected repeatedly in the ash of tobacco plants by the effects of these metals on the growth of *Aspergillus niger*.

nutrient solution, proved to be due to the removal of zinc and of molybdenum. That the procedures adopted are comparable to those recommended by others for work of this character would seem certain, since as the result of their employment the nutrient solution proved entirely incapable of supporting the growth of various *Rhizobia* and *Saccharomycetes* except upon the addition of other substances.

On the other hand, the use of organic materials or of substances derived from such materials may lead to exaggerated responses because of the concomitant addition of heavy metals with the supposed accessory growth factors. The presence in such materials of heavy metals essential for growth is definitely indicated in these experiments and should be taken into account. The employment, for example, of a culture medium considered by Allison and Hoover (*1*) to be suitable for demonstrating the necessity of "coenzyme R" for *Rhizobia* involves the use of an excess of calcium salt at pH 6.9 from which the clear solution is siphoned. This procedure happens to be a variant of the writer's method of nutrient-solution purification for the removal of heavy metals. Utilization of this treatment suggests the partial influence of heavy metals on the increases in growth attributed to "coenzyme R."

The absence of evidence in confirmation of the results of Nielsen and Hartelius (*6*) with *Aspergillus niger* and "growth substance B" requires further elucidation. It may be, of course, that the strain employed by Nielsen and Hartelius would respond exactly as does the "W" strain under these conditions. This might imply that the hypothetical "growth substance B" is simply a mixture of essential heavy metals, perhaps in organic combination. On the other hand, there is definite evidence in the literature that the individual accessory growth substances are required in markedly different degree even by forms in closely related genera. That this would also apply to species of the same genus and even to strains of the same species in some cases is not improbable. Another interpretation is possible, however. Since the necessity for these specialized bodies would imply a loss in physiological functions, namely, the ability to synthesize various organic chemical nuclei essential for metabolism, its effect would be to induce specialization within a more or less restricted habitat. That is to say, an organism like *Aspergillus niger*, which seems to grow quite freely on all types of substrata, would not be apt to require accessory growth substances unless these are universally present in practically all types of organic materials.

SUMMARY

The growth and development of *Aspergillus niger* in synthetic nutrient solutions have been studied with special reference to the influence of heavy metals and accessory growth substances. The results indicate that accessory growth substances are not required for the nutrition of *A. niger* ("W" strain). The decreases in yield of this fungus consequent upon utilization of sucrose purified by alcohol (the procedure for removal of "bios" and "coenzyme R") are due almost entirely to the removal of zinc and of molybdenum. Extracts of organic materials, also, such as yeast decoction or malt extract, when used as a source of assumed accessory growth substances, may cause an increase in growth because of the essential heavy metals they contain.

Molybdenum is essential for the growth and development of *Aspergillus niger*, and therefore cannot be associated exclusively with the process of nitrogen fixation in bacteria. An optimum solution (5 percent sucrose) for the growth and development of this fungus, containing iron, zinc, copper, manganese, and molybdenum, as well as the usual constituents, has a total salt content of 2.50 g per liter. Experimental precision within 1 percent in the values of percentage maximum yield is attainable with duplicate cultures in replicate trials.

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CHRYSIS SHANGHAIENSIS SMITH, A PARASITE OF THE ORIENTAL MOTH[1]

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INTRODUCTION

Chrysis (*Pentachrysis*) *shanghaiensis* Smith is a parasite of the oriental moth, *Cnidocampa flavescens* (Walk.) (*3*),[3] in the Orient, its native habitat, but attempts to establish it in the area around Boston, Mass., infested with this moth have been unsuccessful. These attempts were made by Fernald (*5*) in 1917 and 1918, when he liberated adults that issued from cocoons of the oriental moth parasitized by *C. shanghaiensis* received from China. Although 6 percent of the cocoons collected in 1919 were parasitized, no recoveries from these liberations have been made since that time.

In recent years several shipments of oriental moth cocoons have been made to this country from Japan with the object of introducing parasites that might aid in the control of this pest. Among those received in 1932 was *Chrysis shanghaiensis*.[4] This paper presents the results of two seasons' work with the species and notes on its habits. It was intended to conduct experiments the following year, but the low temperatures in the winter of 1933–34 killed all the hibernating larvae of *Chrysis*.

GENERAL HABITS OF CHRYSIDIDAE

The majority of the chrysidids are parasites or inquilines of solitary wasps and bees. The females lay eggs in the nests being provisioned by the rightful owner, and the larvae developing from these eggs eat the provisions, or the larvae of the wasps and bees that develop on the provisions, or both.

Maréchal (*12*), describing the development of *Chrysis ignita* L. on *Odynerus parietum* L., stated that the larva of *Chrysis* consumes the partly developed larva of *Odynerus* and the remaining provisions in the nest.

Ferton (*6, ch. 5*) observed that some species of *Chrysis* prevent competition from their own kinds by the destruction of all eggs by the first larva to hatch.

Hicks (*8*) described the habits of *Chrysis pacifica* Say, parasitic on the mature larvae of *Alcidamea brachyodonta* Ckll. The host cell is stored with pollen. The larvae of *Chrysis* were found on host larvae

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[2] The author is indebted to C. W. Collins, in charge of the laboratory of the Bureau of Entomology and Plant Quarantine, formerly at Melrose Highlands, Mass., for making these studies possible, and to C. P. Clausen, in charge of foreign parasite introduction, of the same Bureau, for abstracts of papers concerning the habits of Chrysididae.

[3] Reference is made by number (italic) to Literature Cited, p. 457.

[4] These cocoons were sent to the Bureau of Entomology laboratory at Melrose Highlands, Mass., by L. B. Parker, of the Japanese and Asiatic beetle investigations, who had collected them on the island of Shikoku, Japan.

of various sizes, but little or no feeding occurred until the host reached the prepupal stage.

Janvier (*9, p. 287*), working with *Tetrachrysis carinata* Guer., an external parasite of the mature larva of *Odynerus humeralis* Hal. in Chile, found that feeding host larvae are not attacked but only those that have spun their cocoons. The male *Tetrachrysis*, after feeding for several days on flowers, searches out a host cell and makes a hole in it. It is then revealed that this cell contains a female *Tetrachrysis*. The female is seized by the thorax and pulled out and mating is then accomplished.

Chapman (*2*) recorded observations on *Chrysis bidentata* L. (*viridula* L.), a solitary, external parasite of the larva of *Odynerus spinipes* (L.) Spin. This species attacks mature larvae of the host in cells that are incompletely protected in unfinished burrows. From 6 to 10 eggs are placed on a host larva, but there is no evidence that more than 1 egg ever hatches.

Bordage (*1*), observing the development of *Chrysis lusca* var. *concinna* Grib., a parasite in the cells of *Sceliphron*, noted that the egg of *Chrysis* hatches after the host egg hatches and that a combat occurs between the primary stages of the parasite and the host. If the host egg does not hatch, the parasite larva dies without touching the mass of food available.

Maneval (*11*) cited the only instance of internal parasitism recorded for Chrysididae. *Chrysis neglecta* Shuck. is a solitary, internal parasite of the larva of *Osmia villosa* Schenck. The first-instar larva of *Chrysis* enters the host larva and develops as an internal parasite.

Chrysis shanghaiensis is an exceptional chrysidid, as it is a parasite of a lepidopterous host. The writer has seen no reference to any other chrysidid acting as a parasite of a lepidopteron.

LITERATURE ON CHRYSIS SHANGHAIENSIS

Chrysis shanghaiensis was described in 1874 by Smith (*15*). Mocsáry (*13, p. 522*) listed *shanghaiensis* as the only chrysidid parasitic on a lepidopterous host. Joannis (*10*) noted the presence of *C. shanghaiensis* in cocoons of the oriental moth received from China from P. R. Gaudissart, a missionary. The adults were determined by Gribodo, who published notes (*7*) on some of the habits of the species which he observed in material received from Joannis. Du Buysson (*4*) presented the distribution of the species, described the poison glands of the female, and synonymized *C. hymalaiensis* Mocs. and *C. crassicuta* Mocs. with *C. shanghaiensis*. The early attempts of Fernald (*5*) to establish *C. shanghaiensis* in the area around Boston infested with the oriental moth have already been mentioned. Piel and Covillard (*14*), in a paper on the oriental moth and its parasites, devoted considerable space to a discussion of *C. shanghaiensis*, making many valuable observations on the habits of the species and describing some of the larval and pupal development.

DISTRIBUTION

According to Piel and Covillard (*14*), *Chrysis shanghaiensis* is a southern form, and they recorded it from India, the Himalayas, China, Siam, Sumatra, Java, and Gorontalo. It is also found in

Japan. *Cnidocampa flavescens* is recorded from the Orient, eastern Siberia, China, Chosen, and Japan. Since the range of *C. shanghaiensis* does not coincide with that of *C. flavescens*, it is probable that this parasite can reproduce on other Eucleidae found in these regions.

HABITS

BEHAVIOR OF ADULTS

Adults of *Chrysis shanghaiensis* respond quickly to changes of light and temperature. During cool weather or in subdued light they congregate in places that afford protection and concealment. In the laboratory they are found between the cloth of the cage and its wooden framework, beneath papers upon which the food is placed, or in empty host cocoons. In bright light and at higher temperatures their activity increases. The antennae vibrate continuously, and at intervals the wings produce a rather high pitched buzzing sound. This is accomplished without spreading the wings and is apparently due to a rapid friction between them. The adults are capable of strong and rapid flight.

Like the adults of various other Chrysididae, they defend themselves against attack by wasps or bees by curling the abdomen under the thorax so that no soft parts of the body are presented to the sting of the aggressor.

MATING

Adults of *Chrysis shanghaiensis* mate readily in cloth cages, or in wooden boxes with glass tops, on clear, warm days, and also, although more slowly, when there is no sunshine. The males are very active and pursue the females vigorously, vibrating their wings so rapidly that they appear as a blur. This causes a humming sound that can be heard several feet away. In 15 observed matings the act lasted from 6 to 14 minutes, the average being 8 minutes. None of the females mated more than once, but two males mated twice. Attempts to obtain further mating by these males were unsuccessful.

METHOD OF ATTACK

The cocoons that were attacked in the laboratory had been collected in the field by cutting the twigs upon which they were spun, or had been formed in the laboratory by last-instar larvae collected in the field. Cocoons on twigs of the diameter of a pencil or larger were attacked most readily.

The female *Chrysis* approaches a cocoon and examines it with her antennae. If it proves satisfactory, she takes a position on the twig facing either end of the cocoon. If the cocoon has been spun in a crotch formed by two twigs, she can attack the cocoon from the side, but normally she prefers the ends. She grasps the twig firmly, the posterior legs slightly arched and the anterior legs placed somewhat forward, and applies the mandibles about one spot on the cocoon. While chewing a hole in the cocoon, she braces the five pointed projections on the tip of her abdomen against the surface of the twig. She holds her body rather rigidly, but as the mandibles are scraping about the point of attack she turns her head first one way and then the other, making striations that completely surround the central point. When a hole is broken through the cocoon, she places the point of one

mandible in the hole and that of the other firmly on the surface of the cocoon away from the hole. The action then is a rasping and pulling one, working from the center toward the mandible which is used as an anchor.

In seven instances this operation required from 10 to 30 minutes. In the majority of cases observed, however, it consumed nearly 30 minutes, the 10-minute period being exceptional.

The wall of the host cocoon is strengthened by a gradual hardening of the cementing substance used in its formation. It was noted that cocoons used a few hours after the host larva had completed the spinning were not satisfactory for *Chrysis*. Instead of chewing a small, round hole, the parasite would tear a jagged hole in the cocoon and leave without ovipositing. This did not occur when cocoons were held for 24 hours or more before they were placed with the *Chrysis*.

OVIPOSITION

In ovipositing, the female reverses her position on the twig and faces away from the cocoon. The long ovipositor is exserted, and she attempts to locate the hole that she has made in the cocoon. In a few of the cases observed the hole was apparently not suitable, for the female turned and enlarged it. When the ovipositor is fully inserted, the tip of the abdomen is practically resting on the cocoon.

The ovipositor of *Chrysis* is about 7 mm long and is very flexible. Observations made through an opening in the side of a cocoon showed that the female extends the ovipositor around the larva on all sides before depositing an egg. Apparently poison is injected during this prodding, for the host larva becomes paralyzed. The act of egg deposition requires from 6 to 30 minutes, the average duration being 15 minutes.

After depositing the egg, the female again applies her mandibles to the cocoon and scrapes the surface toward the hole. The loosened material she mixes with an oral secretion and packs into the hole, sealing it. The area surrounding the sealed hole is marked with fine radiating striations to a distance of about 1 mm. The sealing of the hole requires from 9 to 37 minutes, the average being 20 minutes. Rarely a female will leave the cocoon without sealing the opening.

EGG CAPACITY

Seven females deposited 79 eggs from August 26 to November 7 under laboratory conditions, or an average of 11.3 eggs per female. This seems to be a small number for such a numerous species, but it is slightly larger than the 8- to 10-egg average determined by Piel and Covillard (*14*) for the group of females that they studied. It is possible that this species deposits more eggs under natural conditions.

DEVELOPMENT

EGG

The egg of *Chrysis shanghaiensis* (fig. 1) is deposited externally on the body of the host larva within its cocoon and adheres only slightly unless it is deposited on the venter, which in itself has adhesive qualities.

A freshly deposited egg is dull white, elongated, rounded, and slightly curved, bearing a very short petiole at the cephalic end. The surface is finely roughened by crystal-shaped protuberances, which are more clearly seen on an egg from which the larva has hatched.

Measurements of 10 eggs showed them to range from 2.1 to 2.5 mm in length and from 0.4 to 0.5 mm in width. Hatching occurs about 4 days after deposition.

FIRST-INSTAR LARVA

The newly hatched first-instar larva (fig. 2) measures 1.5 to 1.9 mm in length and 0.5 to 0.6 mm in width. It consists of a large, squarish head and 13 body segments which, viewed laterally as in figure 2, vary slightly in width for two-thirds the length of the body, then taper off to a bluntly bifurcate posterior segment which is used as an aid to locomotion. The body is white at first, but it soon becomes creamy yellow from the ingested food. The pleura bear fleshy lobes.

FIGURE 1.—Outline drawing of an egg of *Chrysis shanghaiensis*. × 20.

The endoskeleton of the head, upon which the mandibles articulate, is well sclerotized. The labrum, labium, and maxillae are fleshy and prominent. The mandibles are shown in figure 3, *A*.

The tracheal system consists of two lateral trunks starting in the prothoracic segment and extending posteriorly, where they are joined in the twelfth body segment. The spiracles are present in nine segments, the mesothoracic and the first eight abdominal segments. In the metathoracic segment there are well-developed spiracular branches but no apparent spiracular openings.

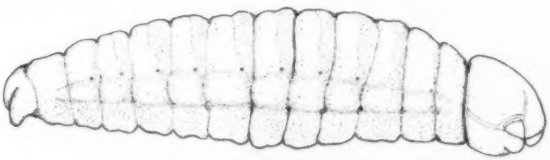

FIGURE 2.—Partially grown first-instar larva of *Chrysis shanghaiensis*. × 63.

The first stadium averages 4 days in length.

INTERMEDIATE INSTARS

There are three intermediate instars. The exuviae can be found just behind the feeding larva or still attached to it. The changes in the body in these instars are slight. It becomes relatively stouter, the fleshy lobes on the pleura become more pronounced, and the last segment loses its bifurcate character with the first molt. The head of the larva becomes more hemispherical with each molt and the endoskeleton more heavily sclerotized. The mandibles show considerable change, as illustrated in figure 3, *B*, *C*, and *D*.

In the second instar the metathoracic spiracular opening appears, and in the remaining instars there are 10 pairs of open spiracles.

Each of the intermediate stadia averages 3 days in length.

LAST-INSTAR LARVA

When the last-instar larva has completed its growth, there is little left of the host except the sclerotic parts of the head and some body spines. The larva spins a golden silken cocoon, excluding the remains of the host and the meconium. Figure 4 shows an average full-

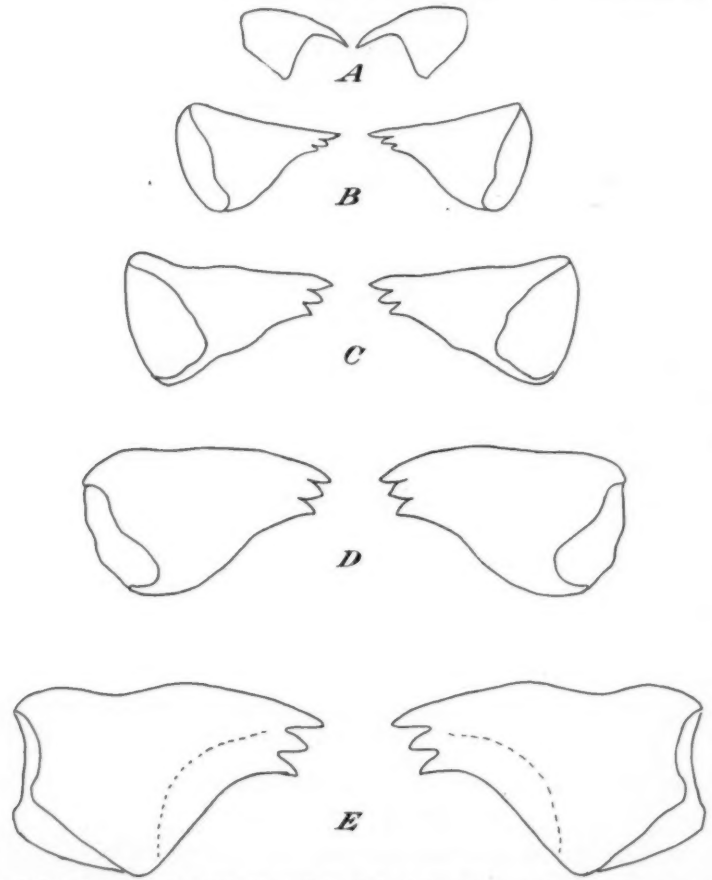

FIGURE 3.—Outline drawings of larval mandibles of *Chrysis shanghaiensis:* A, First instar; *B*, *C*, and *D*, intermediate instars; *E*, last instar. × 112.

grown larva, which measured 10.5 mm in length and 5.2 mm in width. The last-instar larva is creamy yellow in color.

Figure 5, showing the head of a mature larva, is given in some detail, for it is representative of the head characters found in the other instars. The lower part, including the labium and the maxillae, is sharply set off from the upper part, bearing the labrum, the mandibles, and the antennae. The sclerotized features of the labial and maxillary regions of the head are hidden by the fleshy labium and maxillae. The large brownish mandibles are shown in figure 3, *E*.

PUPA

Pupation occurs the latter part of June. The pupa is white at first. The eyes and the ocelli soon darken, and then the body shows a faint greenish reflection which gradually becomes more pronounced

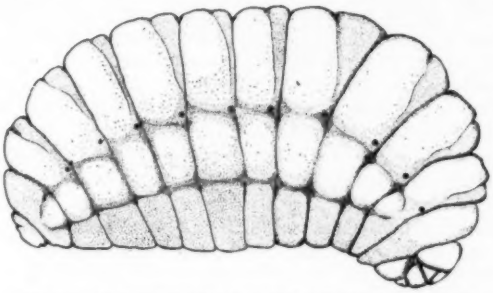

FIGURE 4.—Mature last-instar larva of *Chrysis shanghaiensis*. × 7.

and finally becomes a deep bluish green with iridescent reflections. This gradual coloration progresses from the pronotum and the dorsum of the mesonotum to the metathorax, the abdomen, and finally to the legs. The pupal stage lasts a month or more, the first pupa having been noted on June 26 and the first adult on July 26.

LONGEVITY

The adults of *Chrysis shanghaiensis* live very well in confinement on food consisting of either honey and water (1 to 4) or lump sugar and water, the water being supplied on sponges.

Piel and Covillard (*14*) found that the males lived 12 to 15 days and the females more than a month under conditions in China. In experiments at this laboratory adults lived much longer. In 1932 the issuance of adults started on July 20 and extended to August 4, and host cocoons were attacked from August 11 to September 22. In 1933 the issuance of adults started July 26 and extended to August 24. Aside from two males and one female that died a short time previously, six dead males were removed on October 10, 47 days after the last adult issued. No more dead were removed until October 17, when two females died. The last adult died January 20, 1934. The length of life would be considerably shortened in nature because of the cold weather. This period of survival was possible under laboratory conditions because the adults were placed in a heated insectary. This record includes 97 adults.

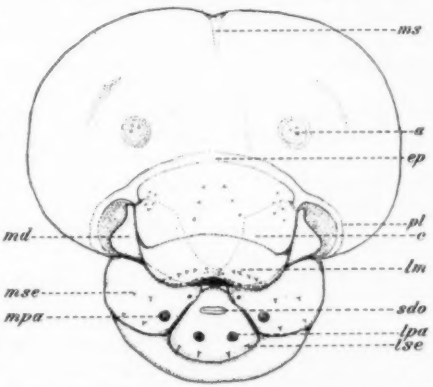

FIGURE 5.—Head of mature larva of *Chrysis shanghaiensis*, × 36; *a*, Antenna; *c*, clypeus; *ep*, epistoma; *lpa* labial palpus; *lse*, labial seta; *lm*, labrum; *md*, mandible; *mpa*, maxillary palpus; *ms*, metopic suture; *mse*, maxillary seta; *pl*, pleurostoma; *sdo*, orifice of silk duct.

SEASONAL HISTORY

Piel and Covillard (*14*) found two generations of both host and parasite in China, the first generation of *Chrysis* appearing in the latter part of June and the second generation in August. In this laboratory the parasites overwintered as mature larvae within the host cocoons, the adults appearing late in July and early in August. The adults mated soon after issuing and attacked the host larvae within cocoons. The larvae became mature about 20 days after egg deposition, and each spun a golden, silken cocoon within the cocoon of its host and remained in this condition throughout the winter.

Chrysis was found in all stages of larval development until cold weather stopped the activity of the adults.

INTERRELATIONS OF CHRYSIS AND CHAETEXORISTA

Studies were begun on the interrelations of *Chrysis shanghaiensis* and *Chaetexorista javana* B. and B., a tachinid parasite of the oriental moth that was established in the infested area in 1929 and 1930, but the extreme cold weather during the winter of 1933–34 killed all the material that was held in hibernation, making it impossible to continue this work.

These incomplete experiments indicated, however, that competition exists between these species. Both species develop on the host larva within its cocoon, *Chaetexorista* internally and *Chrysis* externally. A larva of *Chrysis* feeding externally on a host larva containing a second-instar larva of *Chaetexorista* usually matured at the expense of *Chaetexorista*. In some instances *Chaetexorista* developed into the third instar, which is normally attained after overwintering. This may have been due to the stimulation resulting from the feeding of the larva of *Chrysis*. In such cases the larva of *Chaetexorista* usually matured and formed a puparium, sometimes imperfect or larviform, and *Chrysis* died from lack of food. Some host cocoons were found to contain dead larvae of both species.

FACTORS LIMITING THE EFFECTIVENESS OF CHRYSIS

Piel and Covillard (*14*) discussed a chalcid parasite, *Eurytoma monemae* Ruschka, which depends upon *Chrysis* for its ability to oviposit within a cocoon of the oriental moth. This parasite oviposits through the plug of material with which *Chrysis* seals the opening in the cocoon made during oviposition. These investigators infer that *Chrysis* is overcome by *Eurytoma* in such instances.

At this laboratory several larvae of *Chrysis* in cocoons that the female had failed to seal after oviposition were devoured by small chalcid larvae which proved to be *Mellitobia* sp. It is believed that *Mellitobia* would be unable to oviposit within a normal cocoon.

In December 1933 and early in 1934 the temperature in the laboratory yard was as low as −18° F. This temperature killed all the larvae of *Chrysis* hibernating within host cocoons held in an unheated insectary. During the two previous seasons the lowest temperature was −4° F., which allowed successful hibernation.

SUMMARY

Chrysis (*Pentachrysis*) *shanghaiensis* Smith is a parasite of the oriental moth, *Cnidocampa flavescens* (Walk.), a pest of various shade and fruit trees. The oriental moth is established in and around Boston, Mass. Parasitized material from Japan yielded this chrysidid parasite, and studies concerning its biology were conducted. *C. shanghaiensis* is the only known chrysidid that is a parasite of a lepidopterous host.

The adults of *Chrysis shanghaiensis* are very active and capable of strong flight. The females mate readily and attack the larvae of the oriental moth by chewing a hole in the cocoon, depositing an egg in it external to the host larva, and then sealing the hole. The egg hatches in about 4 days, the larvae complete the first stadium in an average of 4 days, and the second, third, and fourth stadia in an average of 3 days each. The last-instar larva encloses itself in a golden silken cocoon, where it remains throughout the winter. The larva pupates during the latter part of June, and the adults emerge late in July and in the first part of August.

The adults live very well in captivity. Of 97 adults that emerged between July 26 and August 24, only 9 had died 47 days after the last adult emerged. The last adult died on January 20, nearly 5 months from the termination of issuance.

Uncompleted experiments on the interrelations of *Chrysis shanghaiensis* and *Chaetexorista javana* indicated that some competition existed when both species attacked the same host larva.

One case of parasitism at the laboratory was traced to *Mellitobia* sp., but this occurred only because the oviposition hole in the cocoon was not sealed. The low temperatures of the winter of 1933–34 proved too severe for the *Chrysis* larvae held at the laboratory, and no doubt such temperatures would limit the survival of this insect in the vicinity of Boston.

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THE INFLUENCE OF VARYING AMOUNTS OF WATER-SOLUBLE PHOSPHORUS IN DIFFERENT SOIL TYPES ON THE RESPONSE OF CULTIVATED CROPS[1]

By O. C. BRYAN, *professor of soils, Department of Agronomy, University of Florida College of Agriculture,* and W. M. NEAL, *associate in animal nutrition, Department of Animal Husbandry, Florida Agricultural Experiment Station*

INTRODUCTION

A low content of available nutrient elements in the soil is reflected in the growth of the vegetation, and often in the welfare of animals grazing over such areas. Phosphorus appears to be one of the elements deficient in the forage growing on some of the fine sandy loam soils in Florida. Lack of phosphorus results in the condition known as "sweeny" (phosphorus deficiency) in cattle. This condition does not occur on the coarser textured soils of the same locality.

The object of this investigation was to determine the response of different plants to varying amounts of phosphorus in soils of varying texture. Virgin samples of Orangeburg fine sandy loam were used as typical of the phosphorus-deficient soils, while Norfolk sand and Norfolk fine sand were used to represent types on which sweeny does not occur.

REVIEW OF LITERATURE

The content of water-soluble phosphorus in sands was shown by Bryan (*3*)[2] to be much higher than that for sandy loams and loams even under conditions of heavy fertilization. This was due to the greater amounts of phosphate-fixing agents, iron and aluminum, in the finer textured soils and was intensified by the removal of soil bases through leaching processes. Because of this high fixing power of most humid soils the range of soluble phosphorus in the soil is limited. This has been shown by a number of investigators (*5*, *7*, *8*), especially with fine-textured soils, even regardless of frequent additions of phosphates.

Becker, Neal, Shealy, and York (*2*) found that the phosphorus content of the native forage plants growing on the fine sandy loams was less than that of the plants growing on coarser textured soils in the same locality. The level of phosphorus in the first instance was less than that required for the welfare of cattle. Theiler, Green, and Du Toit (*11*), Eckles, Becker, and Palmer (*4*), Scott (*9*), and others have found that a deficiency of phosphorus in cattle was due to a deficiency of this element in the forage vegetation. Mather (*6*) showed that phosphate fertilization of sands and peats increased the phosphorus content of the vegetative portion of forage, most of the increase being in the inorganic fraction. The results of Weidemann (*13*) confirm the increase of phosphorus in the vegetative portion when heavy fertilization is practiced.

[1] Received for publication Oct. 1, 1935; issued April 1936. The work reported in this article is one phase of the investigation of phosphorus deficiency in cattle as it occurs in Florida, and not only verifies the deficiency of phosphorus in the forage grown on affected areas, but indicates that excessive phosphate fertilization would be required to produce "protective" forage.

[2] Reference is made by number (italic) to Literature Cited, p. 466.

In the veld soils of South Africa, rainfall is often the limiting factor (*11*) so that phosphate additions on the soils where cattle suffer from a deficiency of the element do not give consistent increases in yields of forage. Stokes, Warner, and Camp (*10*) found phosphate fertilization to give little or no increase in yield of corn on the light, sandy soils of central Florida, while marked increases were secured on the heavier soils of northwest Florida. These latter soils are in the area where cattle suffer from phosphorus deficiency.

In general, fine-textured soils have a low content of water-soluble phosphorus. This may be increased by phosphate fertilization, but to a lesser extent than in the coarser textured soils. Phosphate fertilization may increase the phosphorus content of forage, and thus prevent the deficiency in cattle. But whether or not this would be economical in range soils remains to be shown.

METHOD OF PROCEDURE

The soils were placed in glazed, 2-kg earthenware jars and treated with superphosphate at rates varying from 0 to 8,000 pounds per acre. The cultures also were given a uniform treatment of 500 pounds of gypsum and 200 pounds of dolomitic limestone per acre. These materials were mixed thoroughly with the soils, which were allowed to stand for 12 weeks with weekly additions of water, in order to permit the soil and the amendments to reach equilibrium. At the end of the 12 weeks, nitrogen was added at the rate of 100 pounds each of nitrate of soda and calcium nitrate, and potash as sulphate at the rate of 75 pounds per acre. Mustard (*Brassica*), vetch (*Vicia*), and sorghum (*Holcus*) were used to measure the availability of the phosphorus in the soils. All series were replicated.

Mustard was planted first and water added as needed. During the growth of the crop, soil samples were taken at three intervals, and the water-soluble phosphorus was determined colorimetrically in 1 to 5 soil-water extracts (*12*). At the end of 60 days, the plants on each culture were cut, dried, and weighed.

Following the removal of the mustard crop, the soils in each jar were mixed, the fibrous roots removed, additions of nitrogen and potash made, and the cultures planted to vetch. The crop was allowed to grow, as in the case of the previous crop of mustard. The water-soluble phosphorus was also determined as before. At the end of 4 months the vetch was cut, dried, and weighed, and the phosphorus content determined by standard methods (*1*).

Following the crop of vetch, the soils were again mixed; potash and nitrogen were added as before; and sorghum was planted. However, the Norfolk sand was omitted. This crop was grown for 60 days, before the photographing and the taking of growth records. The phosphorus content of sorghum was determined as in the case of vetch.

For comparison a second series of soils was planted to sorghum, Norfolk fine sand and Orangeburg fine sandy loam being used. As with the other cultures, superphosphates were added in varying amounts, but in this case the seed was planted immediately following the application of the phosphate. Nitrogen and potash were added and the plants allowed to grow for 40 days.

RESULTS

The oven-dry weights of mustard and vetch as affected by the water-soluble phosphorus in the soils are given in table 1. All values given are the average of two or more replications. The relative growth of all the crops with different amounts of phosphorus is shown in figures 1, 2, and 3. Examination of the data shows that the response of mustard and vetch to phosphates on Norfolk sand was small as compared with the response on the Orangeburg fine sandy loam. The response to phosphorus on the Norfolk fine sand was intermediate between that obtained on the other soils. The growth of mustard increased with increase in phosphate applications up to 1,000 pounds

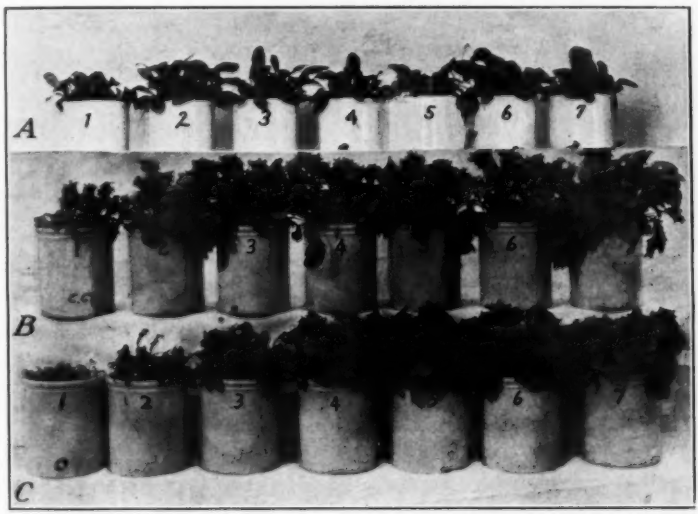

FIGURE 1.—The effect of different amounts of phosphorus upon the growth of mustard in: *A*, Norfolk sand; *B*, Norfolk fine sand; *C*, Orangeburg fine sandy loam. All cultures treated alike except for phosphorus additions. Nos. 1 to 7 received 0, 500, 1,000, 1,500, 2,000, 4,000, and 8,000 pounds of superphosphate per acre, in numerical order.

per acre on Norfolk fine sand, while the growth increased on Orangeburg fine sandy loam up to 4,000 pounds per acre. It appears that a concentration of 1 part per million of water-soluble phosphorus (1 to 5 soil-water extract) will permit maximum growth of mustard. This level was maintained with approximately 500 pounds per acre of superphosphate on Norfolk fine sand, and 4,000 pounds per acre on Orangeburg fine sandy loam.

The data in table 1 show that the Orangeburg fine sandy loam has a much higher phosphate-absorbing power than either the Norfolk sand or the Norfolk fine sand. A better growth of vetch was obtained on the Norfolk soils without phosphates than on the Orangeburg soil with a 4,000-pound application of superphosphate per acre. A minimum of 0.5 parts per million of water soluble (1 to 5 soil-water extract) phosphorus was required to produce appreciable growth, while increased amounts produced an increase in growth until a level

of about 2 parts per million was reached. This level was higher than that required for mustard. The phosphorus content of the vetch and sorghum as shown in table 2, in general paralleled the water-soluble phosphorus in the soil. The highest phosphorus concentration attained in vetch was 0.75 percent P_2O_5 when grown on Norfolk sand with 15.8 parts per million of water soluble phosphorus. The greatest concentration of water-soluble phosphorus in the Norfolk fine sand was approximately 8 parts per million while that for the Orangeburg fine sandy loam was 0.9 parts per million (table 1). But the phosphorus content of vetch from these two soils was less than that on the Norfolk sand (table 2).

TABLE 1.—*Yield of mustard and vetch grown on three soil types as influenced by different amounts of water-soluble phosphorus*

Crop and acre rate of superphosphate applied (pounds)	Water-soluble phosphorus in extracts			Oven-dry yield of crops from—		
	Norfolk sand	Norfolk fine sand	Orangeburg fine sandy loam	Norfolk sand	Norfolk fine sand	Orangeburg fine sandy loam
Mustard:[1]	*P. p. m.*	*P. p. m.*	*P. p. m.*	*Grams*	*Grams*	*Grams*
0	0.9	1.0	0.20	2.4	2.4	0.3
500	1.8	1.4	.28	2.4	3.5	1.1
1,000	2.3	1.8	.36	2.5	4.1	2.3
1,500	5.8	2.2	.39	2.5	4.3	3.7
2,000	7.8	2.9	.58	2.4	4.4	4.1
4,000	9.6	4.3	.85	2.6	4.0	4.5
8,000	17.2	10.3	1.90	2.4	4.5	4.2
Vetch:[2]						
0	.6	.4	[3]	1.8	1.5	.62
500	.9	.4	[3]	1.6	1.4	.41
1,000	1.2	.8	[3]	2.0	1.4	.35
1,500	2.2	1.6	.12	2.2	2.1	.51
2,000	3.2	2.7	.25	2.2	2.2	.90
4,000	5.8	3.1	.58	2.7	2.7	1.90
8,000	15.8	8.1	.90	2.1	2.5	2.40

[1] Superphosphate mixed with the soil 4 months fallow; planted to mustard; harvested after 60 days.
[2] Vetch followed mustard without additional superphosphate, but with additions of nitrogen and potash; harvested after 120 days.
[3] Trace.

TABLE 2.—*Phosphorus content of vetch and sorghum grown on three soil types as influenced by rate of phosphate fertilization*

Crop and acre rate of superphosphate applied (pounds)	Phosphorus content of crops from—			Crop and acre rate of superphosphate applied (pounds)	Phosphorus content of crops from—		
	Norfolk sand	Norfolk fine sand	Orangeburg fine sandy loam		Norfolk sand	Norfolk fine sand	Orangeburg fine sandy loam
Vetch:[1]	*Percent*	*Percent*	*Percent*	Sorghum:[2]	*Percent*	*Percent*	*Percent*
0	0.114	0.092	0.087	0		0.083	0.070
500	.109	.087	.092	500		.074	.083
1,000	.114	.100	.087	1,000		.100	.079
1,500	.118	.114	.087	1,500		.105	.092
2,000	.170	.153	.096	2,000		.144	.100
4,000	.249	.197	.118	4,000		.201	.105
8,000	.328	.280	.157	8,000		.223	.157

[1] Vetch followed mustard without additional superphosphate, but with additions of nitrogen and potash; harvested after 120 days.
[2] Sorghum followed vetch without additional superphosphate, but with additions of nitrogen and potash; harvested after 60 days.

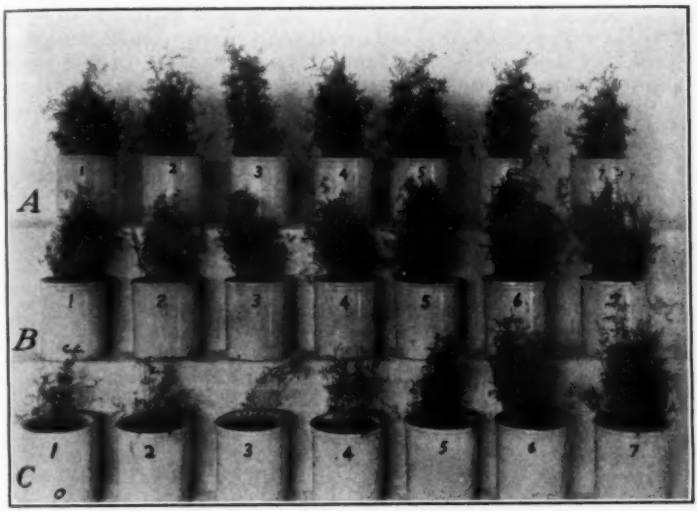

FIGURE 2.—The effect of different amounts of phosphorus on the growth of vetch in: *A*, Norfolk sand; *B*, Norfolk fine sand; *C*, Orangeburg fine sandy loam. All cultures treated alike except for phosphorus additions. Nos. 1 to 7 received 0, 500, 1,000, 1,500, 2,000, 4,000, and 8,000 pounds of superphosphate per acre, in numerical order.

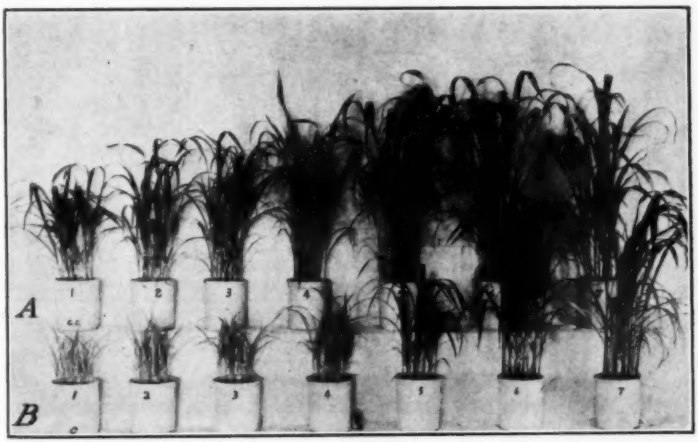

FIGURE 3.—The residual effect of varying amounts of phosphorus on the growth of sorghum in: *A*, Norfolk, fine sand; *B*, Orangeburg fine sandy loam. The sorghum represents the third crop following the application of phosphorus. Nos. 1 to 7 received 0, 500, 1,000, 1,500, 2,000, 4,000, and 8,000 pounds of superphosphate per acre, in numerical order.

The residual effects of the phosphate on the third crop are shown in figure 3, as compared with the initial effects in figure 4. These figures show that when sorghum was the initial crop, maximum growth occurred on the Norfolk fine sand at an application of 500 pounds of phosphate per acre, while additional increments on the Orangeburg soil up to 2,000 pounds produced additional increments of

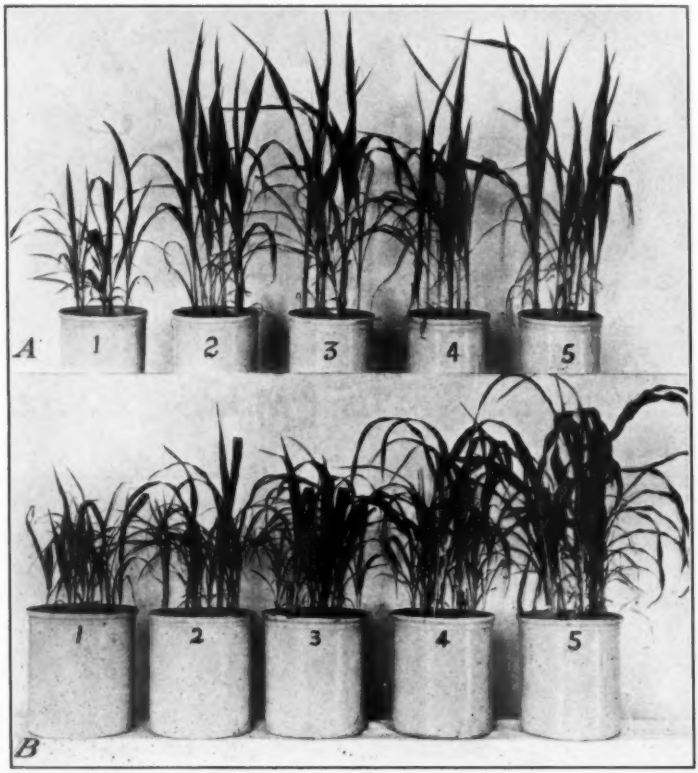

FIGURE 4.—The initial effect of varying amounts of phosphorus on the growth of sorghum in Norfolk fine sand (*A*), and Orangeburg fine sandy loam (*B*). All cultures treated alike except for phosphorus additions. Nos. 1 to 5 received 0,500, 1,000, 1,500, and 2,000 pounds of superphosphate per acre, in numerical order.

growth. But when sorghum was the third crop, the original application of phosphate had become depleted or absorbed by the soils to such an extent that it required a 2,000-pound application for maximum growth in the Norfolk soil and 4,000 pounds in the Orangeburg soil.

The residual effects of the phosphate on the phosphate content of sorghum are also shown in table 2. Here, as in the case of the crop of vetch, the phosphorus content of the sorghum increased with the

content of water-soluble phosphorus in the soil. The results indicate that sorghum responds to phosphate fertilizers equally as well as vetch, but mustard apparently does not respond to phosphates as readily as do either of the other two crops.

DISCUSSION OF RESULTS

The response of the three crops to phosphate fertilization on these representative soils bears out expectations. The Orangeburg fine sandy loam would naturally fix the phosphates more completely than either the Norfolk sand or fine sand, because of the difference in fixing agents (silt and clay) even where lime is applied. This fixation was indicated by the difference in water-soluble phosphorus, growth of the plants, and phosphorus content of the plants.

Under the conditions of this study it appears that a concentration of water-soluble phosphorus (1 to 5 soil-water extract) equivalent to 0.5 part per million was required to secure appreciable crop growth. Increased concentration produced increased growth until a level of approximately 2 parts per million was reached. The phosphorus content was in general proportional to the water-soluble phosphorus in the soil but the general level was less in the Orangeburg soil than in the Norfolk soils.

The phosphorus content of the plants is comparable to that obtained by Becker, Neal, Shealy, and York (*2*) in wiregrass growing on areas of these soils. Five samples of wiregrass from the Orangeburg soils average 0.082 percent of phosphorus while eight samples from the unaffected areas averaged 0.133 percent. During the investigation of nutritional anemia, 35 samples of wiregrass from sands, fine sands, and loamy sands, representing sweeny-free areas, averaged 0.159 percent of phosphorus, according to Becker, Neal, Shealy, and York (*2*).

Because of the crowded condition of the roots in the small volume of soil used in this study the level of water-soluble phosphorus for maximum growth in the different soils would probably be different with different volumes of soil, but the response for the different soils would be comparable.

SUMMARY

A study was made in which varying amounts of superphosphate (0 to 8,000 pounds per acre) were added to Norfolk sand, Norfolk fine sand, and Orangeburg fine sandy loam. Mustard, vetch, and sorghum were used as test plants. Water-soluble phosphorus in soil extracts, yields of crops, and phosphorus content of the plants were determined.

The plants did not respond to a concentration of water-soluble phosphorus greater than 2 parts per million. A concentration of 0.5 part was necessary to secure appreciable growth of sorghum. The greatest response to phosphate addition was on the Orangeburg soil. The phosphorus content of the plants was proportional to the water-soluble phosphorus content of the soil. Because of the high fixing power of the Orangeburg soil for phosphorus, larger applications of the element were necessary for maximum results.

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THE FUNCTION OF COPPER IN SOILS AND ITS RELATION TO THE AVAILABILITY OF IRON AND MANGANESE [1]

By L. G. WILLIS, *soil chemist*, and J. R. PILAND, *assistant soil chemist, Department of Agronomy, North Carolina Agricultural Experiment Station*

INTRODUCTION

Early experiments on the peat soils of North Carolina have shown a striking negative correlation between yields of corn and the accumulation of iron in the nodal tissues.[2] Studies of the oxidation-reduction potentials of these soils were undertaken in an attempt to associate the increase in the soluble iron content of the soil with factors favorable to reductiveness. It appears from the data obtained [3] that the soil potential is governed by an equilibrium between oxygen and the products of microbial decomposition of the organic matter of the soil.

While the exact nature of the oxidation-reduction reaction involved is not clearly apparent, it seems that it is chemical and spontaneous rather than biological. According to this concept, equilibrium potentials are dependent on a constantly replenished supply of oxygen and of the reductive product of microbial activity. The concentration of the latter appears to be extremely low, whereas the supply of oxygen is limited only by the factors governing aeration.

THE EFFECT OF COPPER ON SOIL POTENTIALS

Early in the work on this problem it was considered probable that with adequate aeration, the concentration of the reductive component could be decreased by a catalyst that would accelerate the chemical reaction. With an abundant supply of oxygen, therefore, such a catalyst should raise the soil potential, whereas with deficient aeration the potential should be lowered, provided in the latter case the catalyst did not prevent microbial growth.

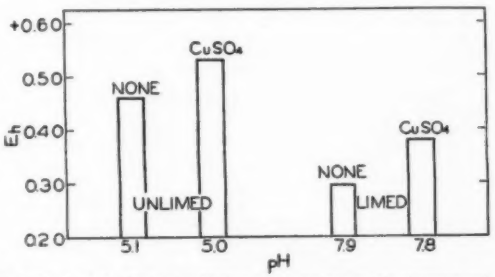

FIGURE 1.—The effect on oxidation-reduction potentials of copper sulphate added to limed and unlimed Dunbar fine sandy loam in aerated water suspension.

Evidence supporting the first of these assumptions is given in figure 1. The soil was a Dunbar fine sandy loam moderately high in organic matter. The copper sulphate was added to a suspension of

[1] Received for publication Oct. 22, 1935; issued April 1936. Published as paper no. 84 of the Journal Series of the North Carolina Agricultural Experiment Station.

[2] SHERWIN, M. E. SOIL TREATMENTS TO OVERCOME THE INJURIOUS EFFECTS OF TOXIC MATERIALS IN EASTERN NORTH CAROLINA SWAMP LAND. Jour. Elisha Mitchell Sci. Soc. 39: 43–48. 1923.

[3] WILLIS, L. G. SOME POTENTIAL CHANGES INDUCED BY LIMING SUSPENSIONS OF A PEAT SOIL. N. C. Agr. Expt. Sta. Tech. Bull. 47, 16 pp., illus. 1934.

the soil which was then shaken in the air for 24 hours and the potentials were determined in equilibrium with air.

The other assumption was verified by noting the potentials of deaerated suspensions of a peat soil without and with added copper sulphate. The results, shown in figure 2, suggest an activation of the micro-organisms in the suspension due to the addition of the copper sulphate, since the negative trend is most rapid with this treatment at the higher pH levels. In the more acid suspensions, however, there is a reversal of the sign of the slope of the potential/pH curve. Similar results have been observed in other work on soil potentials without the addition of copper sulphate and it has been assumed that these can be ascribed to increased activity of other metallic elements as well as copper that may be associated with greater solubilities at the lower pH levels. The type of potential/pH curve obtained with the suspensions containing copper is suggestive of interesting possibilities for further study. Attention has been given recently to the linear character of soil potential/pH curves. Without attempting to expand the discussion beyond the scope of this article, it should be pointed out that this relation holds only where aeration, microbial activity, and other factors contributing to the oxidation-reduction equilibrium can be treated as constant values.

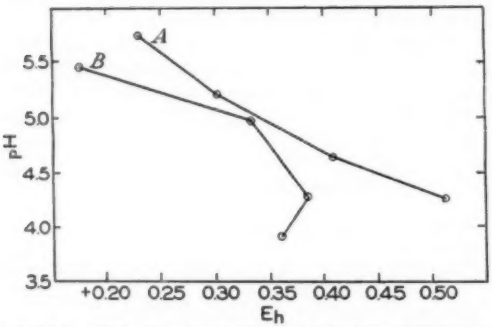

FIGURE 2.—Oxidation-reduction potentials of peat-soil suspensions at different pH levels 9 days after the removal of oxygen: *A*, No copper sulphate added; *B*, copper sulphate added.

Considerable emphasis has been given to the organic-matter content of the soils that have been used in oxidation-reduction studies.[4] These soils have been used largely for the purpose of determining fundamental relationships and trends. In soils of low organic-matter content it is not improbable that the reductive effects of microbial activity are developed only in the rhizosphere.[5] Potentials in such minutely localized zones would not be measurable but they may be expected to exhibit characteristics similar to those found in the more highly organic soils.

It has been suggested[6] that copper acts as an oxidizing catalyst. From the foregoing evidence it appears that this element activates oxidation-reduction reactions in the soil. The mechanism of this activation is not indicated by the results, but in consideration of the effectiveness of relatively small amounts of copper the function would appear to be catalytic.

[4] WILLIS, L. G. See footnote 3.

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EVIDENCES OF COPPER-IRON ANTAGONISM

Much of the evidence to be reported consists of a series of tests of a hypothesis of an antagonistic effect of copper relative to iron. Originally it was assumed that this could be attributed to a direct oxidation of the iron in the soil. Apparently the reactions involved are more complex than this, yet the hypothesis has been of considerable value as a basis for experimentation.

The first experimental test of the hypothesis, in which it was shown that the iron content of corn plants grown on a peat soil could be reduced by applications of copper sulphate, has already been published.[7] A statement of the hypothesis was purposely avoided in that publication pending more supporting evidence. This is furnished by the following observations and data which are presented in sequence.

RESULTS WITH CORN

A field-plot experiment with corn (*Zea mays*) on a peat soil was designed to incorporate all the soil treatments that prior experiments had shown to be favorable. The first crop was virtually a failure on all plots. Between seasons some of the soil was put into pots in the greenhouse so that tests could be made of various supplementary soil treatments. Of these, only copper sulphate gave any evidence of a definite effect. With this compound the seedling corn made a rapid early growth, but the leaves soon became chlorotic between the veins.

An application of a 1-percent solution of ferrous sulphate rubbed lightly on sports on these chlorotic leaves caused a number of them to turn green (fig. 3). Spots rubbed in a similar manner with distilled water did not change, nor was there any effect noted where manganous sulphate was applied alone or with the ferrous sulphate.

It was evident that the application of copper sulphate to the soil produced an iron-deficiency chlorosis. Since the copper sulphate was applied in a thin layer midway of the depth of the soil in the pots, it does not appear reasonable to conclude that the copper modified the properties of the soil mass. These results raise the question whether the copper was effective in the soil or in the plants and also whether the manganese had an effect similar to that of copper.

RESULTS WITH COTTON

Later a series of solution culture experiments was started with cotton (*Gossypium hirsutum*). Copper, zinc, manganese, and boron were added uniformly from a stock solution to all the cultures in amounts supplying 0.1 mg of each of the elements per liter of solution. Iron was supplied as ferric citrate. In the first attempt a severe iron-deficiency chlorosis developed in the fifth leaf of the plants. The work was repeated with the omission of the copper-zinc-manganese-boron solution until the plants were 10 inches high. No chlorosis developed until the plants were over 2 feet high. Then the terminal leaves became chlorotic in the cultures supplying nitrogen as sodium nitrate. The use of the copper-zinc-manganese-boron solution was discontinued and the affected leaves became green within 4 days.

[7] WILLIS, L. G., and PILAND, J. R. THE INFLUENCE OF COPPER SULPHATE ON IRON ABSORPTION BY CORN PLANTS. Soil Sci. 37: 79–83, illus. 1934.

Obviously no question of the oxidation of iron is involved in this case. While the use of the four-element stock solution was a matter of convenience, it was assumed that the copper salt was the significant component. Attention was therefore centered on this element without prejudice toward a possibility of a similar effect of the others. Possibly the copper effected the decomposition of the citrate radical, or the inactivation of the iron may have taken place within the plant. Another possibility carries the implication that the iron of ferric

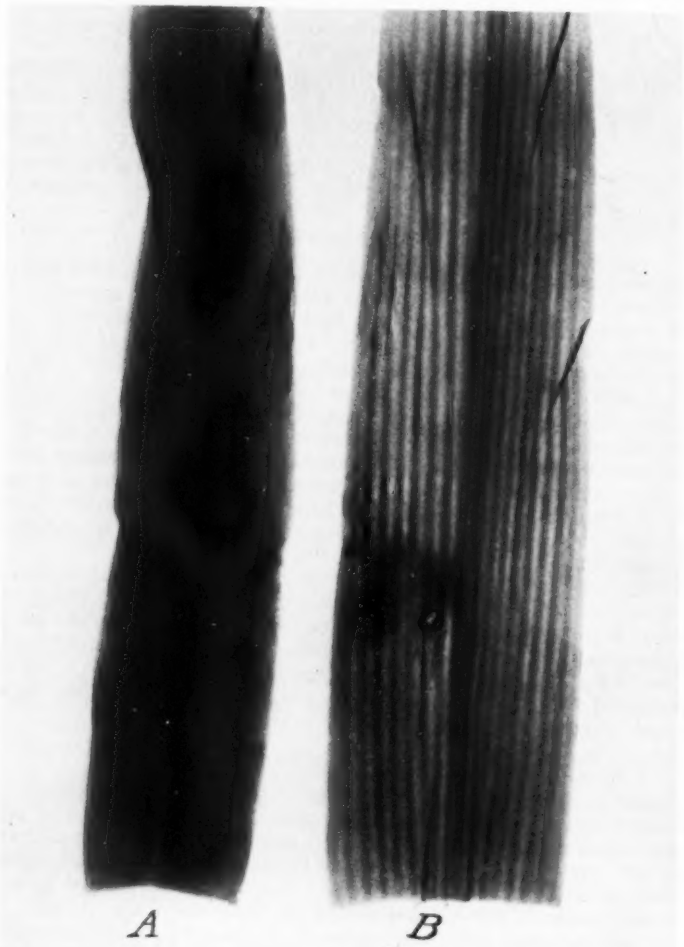

FIGURE 3.—Iron-deficiency chlorosis produced in corn leaves by the addition of copper sulphate to a peat soil: *A*, leaf of plant grown in soil without treatment; *B*, leaf of plant grown in soil to which copper sulphate was added, a green area produced by the external application of ferrous sulphate solution being shown at *a*.

citrate must be reduced before it becomes assimilable by plants and that copper prevents this reduction. This point must be verified before it can be given serious consideration.

The delayed onset of the iron-deficiency chlorosis in the solutions supplied with sodium nitrate occurred at the time that blossom buds were beginning to form. Whether or not the effect has any relation to physiological changes developing at this period of growth is debatable, but the difficulties of the translocation of relatively insoluble elements through long stem growth may have a bearing on the effect of copper.

Identification of Copper Effect

In an effort to verify the assumption that the effect of the stock solution could be attributed to copper, some cotton plants that had been grown in solution cultures without copper or manganese were used as indicators. Iron had been supplied as ferric citrate for the early growth of the plants, but later inclusion of iron was discontinued until the plants showed a definite chlorosis symptomatic of an iron deficiency.

The stems were split from the base for a distance of about 2 inches, leaving approximately one-half of the lateral roots on each segment. Nutrient solutions were put into 500-ml Erlenmeyer flasks which were bound together in pairs so that the tops were in contact and at the same level. The plants were transferred to these flasks, one segment being inserted into each of the paired flasks.

The nutrient solution used had the following composition in grams per liter: Monopotassium phosphate, 0.0892; dipotassium phosphate, 0.1140; sodium chloride, 0.0262; potassium sulphate, 0.1550; potassium chloride, 0.1326; magnesium sulphate -7 H_2O, 0.1363; calcium chloride -2 H_2O, 0.0992; sodium nitrate, 0.1900.

In addition to this, ferric citrate and copper sulphate were added as shown in table 1.

Since it has frequently been observed that symptoms of deficiencies of some of the minor essential elements are not correctable in older leaves, all of these were removed at the time the plants were transferred to the flasks.

Within a week it was unmistakably apparent that the leaves of plants in the A series were becoming normally green. In the C series no evidence of recovery was observed, while the plants in the B series were intermediate in color in the early stages of growth. Later the differences between the A and B series became indistinguishable. The contrast in color of leaves from the A and C series is indicated in figure 4. Identification of the iron deficiency was made by spot treatments with ferrous sulphate on the surface of the leaves from the C series (fig. 4, *B*, *a*). It appears from this evidence that the effect of copper is largely external to the plant, but the delayed onset of iron deficiency in the preceding solution-culture experiment suggests the immobilization of iron within the plant as an additional factor.

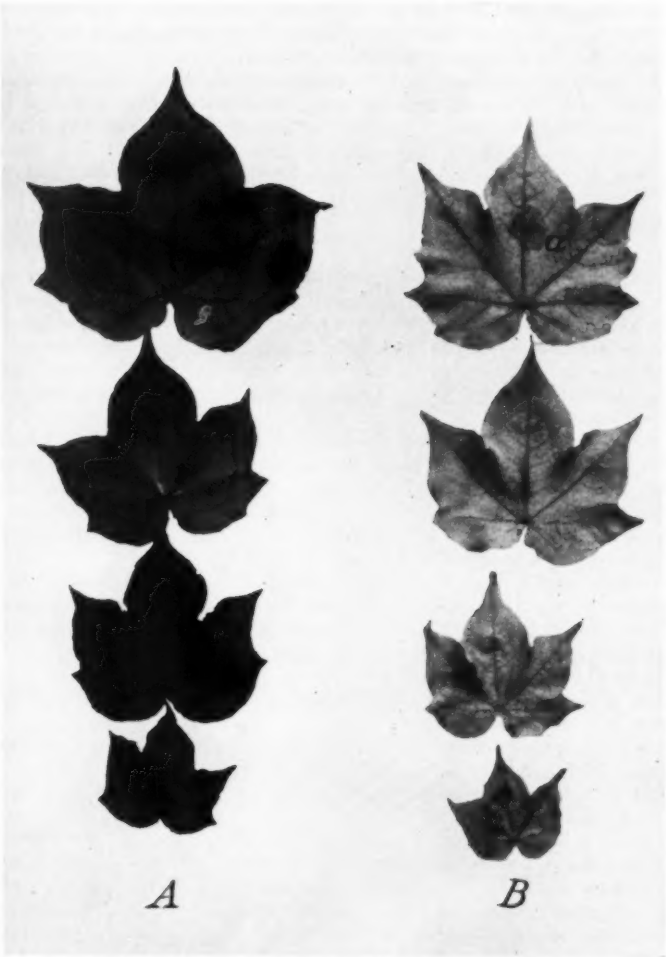

FIGURE 4.—Iron-deficiency chlorosis in cotton leaves resulting from the addition of copper sulphate to culture solutions: *A*, Solutions containing ferric citrate, series A; *B*, solutions containing ferric citrate and copper sulphate, series C, a green area produced by the external application of ferrous sulphate solution being shown at *a*.

TABLE 1.—*Iron and copper additions made to nutrient solutions used in identifying the effect of copper upon cotton plants grown therein*

Series	Flask no.	Addition
A	1	Ferric citrate.
	2	Nothing.
B	1	Ferric citrate.
	2	Copper sulphate.
C	1	Ferric citrate and copper sulphate.
	2	Nothing.

EVIDENCES OF COPPER-MANGANESE ANTAGONISM

Another case bearing on the effect of copper has been given some attention. Fairly extensive areas of soil in the upper Coastal Plain have failed occasionally to produce cotton satisfactorily. Some of this soil was shipped to Raleigh, N. C., and used for pot experiments. The pH value of the soil was 4.9. In the first series of pots it was found that lime was without effect and that fertilizer reduced the growth of the cotton plants. Some benefit was noted where copper sulphate was added to the soil receiving lime and fertilizer with lime.

In a second series of pots, fertilized with ammonium phosphate, ammonium nitrate, and potassium chloride supplemented with varying amounts of calcium sulphate, the best plants were produced on the soils receiving the minimum amount of calcium sulphate. A qualitative analysis of plants from these pots gave strong positive tests for manganese.

Samples of soil and cotton stalks were then obtained in midwinter from fields showing the characteristic damage in varying degrees. The trouble could be correlated with the manganese content of the stalks very closely, as is shown in table 2.

TABLE 2.—*Severity of symptoms in abnormally developing cotton plants as associated with the soil pH and the manganese and iron content (parts per million) of the stalks*

Symptoms	pH of soil	Manganese content of plants	Iron content of plants	Symptoms	pH of soil	Manganese content of plants	Iron content of plants
Slight	4.8	20	44	Moderate	5.5	15	48
Severe	4.6	52	48	Do	5.5	23	62
Slight	4.9	20	25	Do	5.5	29	33
Severe	4.6	53	38	Severe	5.5	30	34
None	5.7	10	20	None		11	23

No significant correlation was found between the poor growth of cotton and either the pH of the soil or the iron content of the stalks.

The soils showing the severest symptoms gave definite qualitative tests for manganese in the water extracts from 5-g samples and stronger tests with the sodium chloride extract.

Further investigation established the fact that the soils involved were invariably found on the second bottom of rivers draining parts of North Carolina and Virginia where soils originating from manganiferous basic igneous rocks are common. It was concluded from the evidence that the manganese found in these soils existed in the bivalent form in the exchange complex and that it was not readily oxidizable to insoluble forms.

It has been found elsewhere in North Carolina that manganese becomes insoluble to the point of deficiency only in soils naturally high in organic matter.[8] A third series of pots was therefore started in which organic matter in the form of finely powdered peat was added. No improvement was observed in the cotton grown on this soil. In fact, the plants grown where lime was added showed abnormal structural deformities characterized by deeply lobed leaves and no

[8] WILLIS, L. G., and MANN, H. B. MANGANESE AS A FERTILIZER. Amer. Fert. 72 (1): [21]-24, illus, 1930.

elongation of the internodes. After 6 weeks it became evident that no further development was being made. At that time 0.1 g of copper sulphate was added in solution to the surface of the soil in each pot. The plants responded noticeably within 2 days and in 2 weeks had become normal in all respects (fig. 5). In no prior case

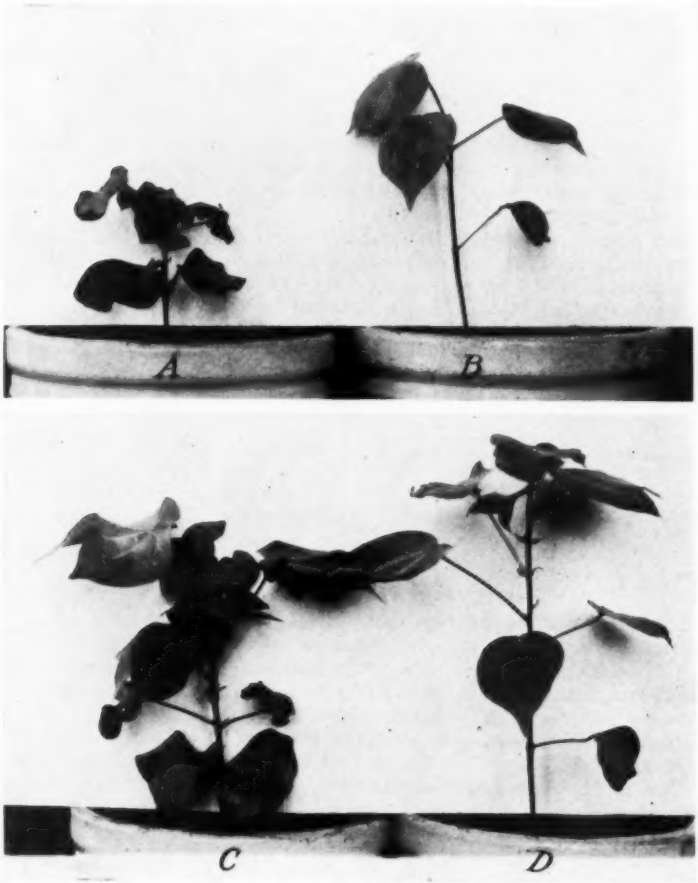

FIGURE 5.—Maximum growth of cotton plants in soil containing an excess of soluble manganese, limed (*A*) and unlimed (*B*), and condition of the same two plants respectively (*C* and *D*) 2 weeks after the addition of copper sulphate.

had any healthy lateral roots developed on any plants. Within 2 weeks after the addition of the copper sulphate, however, the growth of lateral roots had extended throughout the soil in all pots, although it is hardly probably that the copper sulphate had penetrated deeply into the soil.

A fourth attempt at the solution of this problem was made, but in this case cotton grew normally in all the pots irrespective of the treatments. The soil had been stored dry in the greenhouse while the work was in progress and this seems to have eliminated the injurious factor. Inquiries regarding the trouble in the field have brought the unanimous opinion from farmers that crops are damaged severely only in wet seasons.

Although the evidence is not conclusive, there is some support for the assumption that the maintenance of manganese in a state of oxidation in field soils is dependent on the presence of a catalyst such as copper.

SUGGESTED LIMITATIONS TO THE USE OF COPPER SULPHATE IN SOILS

The foregoing results support the conclusion that copper sulphate as it is generally used in soils serves largely as a soil amendment for the correction of conditions under which the excessive concentrations of soluble iron and possibly manganese exist. It is not impossible, however, that there are occasional instances of soils in which copper is deficient in an absolute sense and where this element may be needed as a direct plant nutrient.

It would seem that copper sulphate would be most beneficial when applied to aerated soils having a high content of decomposable organic matter. Where the oxygen supply is limited the effect of copper may be injurious. In well-aerated soils low in total iron and organic matter, copper may cause an iron deficiency except at low pH values. Until these factors have been more completely investigated the indiscriminate application of copper sulphate to soils would appear to be inadvisable.

SUMMARY

Data already published indicate that the oxidation-reduction potential of soils is governed by an equilibrium between oxygen and a chemically active reducing compound which is the product of microbial activity.

It was assumed that this equilibrium could be modified by a catalyst such as copper added to the soil and that this would be reflected in the solubility of the iron compounds of the soil. In preliminary tests it was found that copper sulphate increased the potentials of an aerated soil suspension but decreased the potentials when air was excluded. The significance of these effects was indicated by published evidence that copper sulphate decreases the iron content of corn plants grown on a peat soil.

Corn grown on an unproductive peat soil became chlorotic when copper sulphate was added to the soil. The chlorosis was identified as being due to an iron deficiency when ferrous sulphate applied externally to the leaves caused the development of a green color. The application of manganese sulphate alone or in combination with ferrous sulphate had no visible effect.

Cotton plants grown in solution cultures containing either nitrate or ammoniacal nitrogen and ferric citrate, copper, manganese and zinc sulphates, and boric acid developed an iron-deficiency chlorosis in their early growth. In a repetition of the work with the addition

of manganese, zinc, copper, and boron delayed, a chlorosis developed in the plants receiving nitrate nitrogen when they were 2 feet high. Following the elimination of the four elements from the solution the affected plants rapidly became green.

An experiment in which the roots of iron-deficient cotton plants were divided between two solution cultures has given evidence that copper sulphate will produce an iron-deficiency chlorosis. It appears that this effect is due largely to reactions external to the plant, but there is also evidence of immobilization of iron within the plant under the influence of copper.

Further evidence in support of the assumption that copper serves as a catalyst of oxidation-reduction reactions is presented by a preliminary study of a field soil problem. The soil contained abnormally large quantities of water-soluble and exchangeable manganese. Beneficial effects were observed following applications of copper sulphate or a prolonged drying of the soil.

Copper sulphate serves as a soil amendment, decreasing the availability of iron and possibly of manganese. The effect may be favorable or not depending on the oxidation intensity and the iron and manganese content of the soil.

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